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UNITED STATES DEPARTMENT OF AGRICULTURE

BULLETIN No. 431

Contribution from the Bureau of Entomology
L. O. HOWARD, Chief

Washington, D. C. PROFESSIONAL PAPER February 9, 1917

SACBROOD

By

G. F. WHITE

Expert, Engaged in the Investigation of Bee Diseases

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INTRODUCTION.

Sacbrood is an infectious disease of the brood of bees. It is frequently encountered and has often been the cause of fear on the part of beekeepers through a suspicion that one of the more serious maladies—the foulbroods—was present.

The disease is more benign than malignant. It is insidious in its nature and somewhat transient in its character. The number of colonies that die as a direct result of sacbrood is comparatively small; the loss of individual bees from it, however, in the aggregate is enormous. The loss tends naturally to weaken the colony in which the disease is present, a fact which makes the disease one of great economic importance.

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It will be noted that Jones also recognized that there was a disease that resembled somewhat the genuine foul brood, but was different from it, and that it was also different from chilled, starved, or neglected brood. Most likely the disorder referred to in his article was sacbrood.

Simmins (1887), writing from Rottingdean, England, points out the difference between "deadbrood" and foulbrood:

> That foul brood is often confused with simple dead brood I am well aware. * * * But that every bee keeper may decide for himself without the aid of a microscope, which is the genuine foulbrood and which is not, I will show how I have always been able to detect the difference. With simple deadbrood, while some may appear like the foul disease, much of the older brood *dries up to a white cinder*, in many cases retaining its original form, which I have never found to occur when genuine foulbrood is present. Chilled brood can be distinguished from the more serious malady in like manner.

In addition to emphasizing the difference between "deadbrood" and "foulbrood," Simmins says that these two diseases are in turn to be differentiated from chilled brood. He adds the additional fact also that Cheshire had examined this "deadbrood" and failed to find any microscopic evidence of disease.

Cook (1904), under the heading "New Bee Disease," writes as follows:

> In California and some other sections the brood dies without losing its form. We use the pin-head, and we draw forth a larva much discolored, often black, but not at all like the salvy mass that we see in foulbrood.

From his description, and from the fact that the disease is quite prevalent in California, it is very probable that the disorder mentioned by Cook is sacbrood.

A study of this "dead brood" recognized by the beekeepers as being different from foulbrood was begun by the writer in New York State in 1902, under the direction of Dr. V. A. Moore. In a brief report on the work (1904) the following is found:

> The beekeepers are sustaining a loss from a diseased condition in their apiaries which they are diagnosing as "pickled brood." The larvæ usually die late in the larval stage. The most of them are found on end in the cell, the head frequently blackened and the body of a watery granular consistency. * * *
>
> The results of the examinations showed that *Aspergillus pollinis* was not found. Further investigations must be made before any conclusion can be drawn as to the real cause of this trouble.

It will be observed from this quotation that the so-called pickled brood did not conform to the description of pickled brood and could not therefore be the condition which had called forth the description of and the name, "pickled brood" (see p. 4).

Burri (1906), of Switzerland, writes:

> Dead brood, said to have been black brood, I have occasionally met with in my investigations. It occurred in the older larvæ, and showed a gray to blackish colora-

tion, partially drying the larvæ until mummified. These larvæ of the black-brood type gave a negative result both in microscopic examination and in the usual bacteriological culture experiments. Bacteria seem to take no part in this disease, and so far as I have come in contact with black brood, I have been able to reach no certain opinion as to its cause. [Translation.]

It is very probable that the disorder encountered by Burri, which was free from bacteria, was sacbrood. Out of 25 samples examined between 1903 and 1905, he found four samples containing this disease alone, while in a few of the samples the disorder was accompanied by one of the other brood diseases.

Kursteiner (1910), of Switzerland, gives a summary of all samples examined by Burri and himself from 1903 to 1909. Out of 360 samples of suspected disease examined, 94 were diagnosed as "dead brood free from bacteria." These were probably samples of sacbrood. As shown by his later reports, Kursteiner has continued to find this disease in the examination of suspected samples.

The foregoing references to the literature show that beekeepers in different countries had been observing dead brood in their apiaries which was unlike brood dead of "foulbrood." On this point all of the observers practically agreed. No name had been given to the disorder.

NAME OF THE DISEASE.

Before 1912, very little definite information concerning this somewhat mysterious disorder of the brood had been obtained. After discovering its cause and determining its true nature, the writer (1913) used the name "sacbrood" to designate it. The name was coined to suggest the saclike appearance of the dead larvæ in this disease at the time they are most frequently seen by the beekeeper.

The fact should here be emphasized that sacbrood is not a new disease. It is only the knowledge concerning the disease and its name that is of recent origin. It is far better, and in all probability much more accurate, to think of sacbrood as a disease which has affected bees longer than history records the keeping of bees by man. The disease, therefore, has been collecting its toll of death for centuries, often unawares to the beekeeper. Simply knowing that there is such a disease should not be the cause of any additional anxiety concerning its losses. On the other hand, less fear should be experienced, since by knowing of it hope may be entertained that the losses resulting from it may be reduced.

PICKLED BROOD.

The term "pickled brood" was introduced into beekeeping literature 20 years ago (1896), by William R. Howard of Texas. The condition which he described under this term he declared was caused

by a fungus to which he gave the name *Aspergillus pollini*. In a second article (1898) he writes that pupæ and adult bees, as well as the larvæ, are attacked by the disease, stating his belief that the disease in adult bees had been diagnosed as paralysis. Technically, therefore, the term "pickled brood" refers to an infectious disorder of bees affecting both the brood and adult bees and caused by a specific fungus, *Aspergillus pollini*.

It was particularly unfortunate that these articles on pickled brood should have appeared at the time they did, as through them some beekeepers have been led to the mistaken belief that the brood disease, which they had so long observed as being similar to "foulbrood," but differing from it, had been described in his articles as pickled brood.

Whether such a disease (pickled brood) does exist, can not be definitely stated. It may be said, however, that it probably does not. The writer has not encountered such a disorder during his study on the bee diseases. He believes that if the condition is present it certainly has not attracted the attention of beekeepers to any great extent. It can safely be advised, therefore, that all fear of losses from such a possible condition should be dispelled, at least until the disease is met with again.

It would seem that the name "pickled brood" is being used among beekeepers at present in a very general sense. Root (1913) writes:

> The name pickled brood has been applied to almost any form of dead brood that was not foul brood. In a rather general way, it seems to cover, then, any form of brood that is dead from some natural causes not related to disease of any sort.

This quotation suggests that a number of conditions are most likely included under the term "pickled brood" as it is popularly used. Brood dead of starvation and that found dead before capping and not dead of an infectious disease seem to be referred to especially by the name.

Beekeepers sending samples of disease to the laboratory have been asked the question: "What disease do you suspect?" In the replies received more than one disease was sometimes suggested as being suspected. Out of 189 replies received from beekeepers sending samples of sacbrood, European foulbrood was suggested in 55 replies, pickled brood in 39, foulbrood in 19, blackbrood in 15, poisoned brood in 7, chilled brood in 5, starved brood in 6, American foulbrood in 13, dead brood in 3, neglected brood in 1, scalded brood in 1, suffocated brood in 1, and in 24 cases the reply was: "Don't know." These replies show that beekeepers generally had not learned to recognize the disorder which is now called sacbrood by any one name.

It is natural to suppose that sacbrood would have been one of the conditions occasionally referred to under the term "pickled brood."

As sacbrood has been proved, however, to be a distinct disease and different from all other disorders, naturally it is incorrect to use the terms "sacbrood" and "pickled brood" synonymously, either in the popular or in the technical sense.[1]

APPEARANCE OF HEALTHY BROOD AT THE AGE AT WHICH IT DIES OF SACBROOD.

By comparing the appearance of healthy brood with that of brood dead of a disease, both the description and the recognition of the symptoms of the disease are often materially aided. Before discussing the symptoms of sacbrood, therefore, a description of the healthy brood at the age at which it dies of sacbrood will be given. In this description the same method will be used and similar terms employed as will be found in the description of the symptoms of the disease.

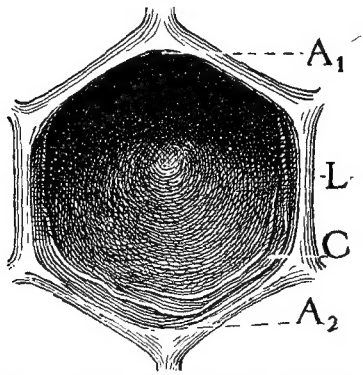


FIG. 1.—Looking into an empty worker cell uncapped by bees. The uppermost angle (A_1), the lowermost angle (A_2), the lateral wall (L), and the wrinkling of the inner surface of the cell near the opening, indicating the presence of a mass of cocoons (C), are shown. Enlarged about 8 diameters. (Original.)

It will be recalled by those who are at all familiar with healthy comb in which brood is being reared that the brood is arranged in such a way that capped and uncapped areas occur alternately and in more or less semicircular fashion. Practically all cells in the uncapped areas will be without caps while practically all in the capped areas will be capped.

Since the brood that dies of sacbrood, with but few exceptions, does so in capped cells, a description of such brood involves the form, size, and position of these cells.

A cell (figs. 1 and 2) may be described as having six side walls, a bottom or base, and a cap. (The cap has been removed by the bees from the cells from which these figures were drawn.) In general the six side walls are rectangular and equal. These walls form six equal obtuse angles within the cell (fig. 1). The angle which is uppermost in the cell (A_1) is formed by two sides which together may be termed the roof of the cell. The angle which is lowermost (figs. 1 and 2, A_2) is formed by two sides which with equal propriety may together be termed the floor of the cell (fig. 2, F). When a cell is cut along its long axis

[1] For the purpose of an explanation for those who may have learned to refer to sacbrood by the term "pickled brood," it might be felt advisable by some to continue for a while in some way a reference to the latter term. In such an event, the expression "so-called pickled brood" is suggested as being more nearly accurate than the term "pickled brood."

the cut surface of the older ones shows the presence of a varying number of old cocoons (fig. 2, C). Near the mouth of the cell on the side walls (figs. 1 and 2, C) will often be noted a wrinkling of the surface. This wrinkling is caused by the presence of old cocoons. The two remaining walls are parallel and will be referred to as the lateral walls (fig. 1, L). The bottom is concave on the inside. The cap

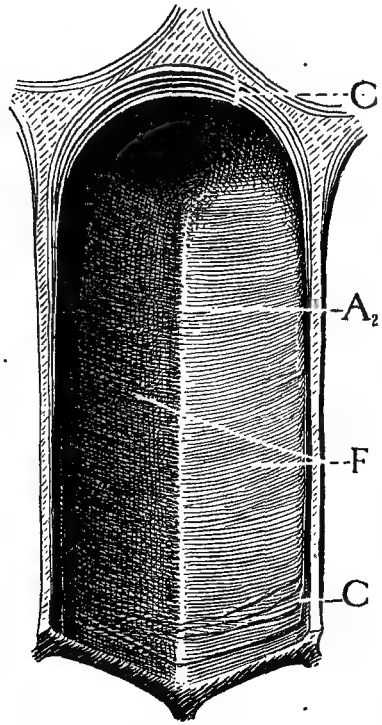


FIG. 2.—Empty worker cell cut in half along the long axis of the cell, showing cocoons (C) at the base and near the mouth of the cell, and the lowermost angle (A_2) formed by the two walls which constitute the floor (F) of the cell. Enlarged about 8 diameters. (Original.)

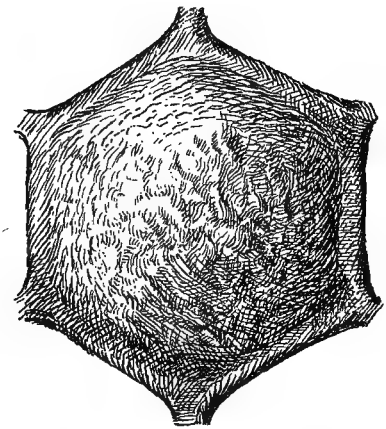


FIG. 3.—End view of cell capped. The cap is convex, being recently constructed. (Original.)

is also concave on the inside, making it convex on the outside.

When freshly constructed the surface of the cap (fig. 3) is smooth and and entire and shows considerable convexity. Later, not infrequently it is found to be less convex and somewhat irregular. The cap should remain normally for the most part entire (fig. 8). While this is the rule, there are exceptions to it. The beekeeper is familiar with the appearance which suggests that it had not been entirely completed (fig. 11; Pl. II, *b*).

The long axis of the cell is nearly horizontal, the bottom of the cell being normally only slightly lower than the mouth. The long axis measures approximately one-half inch, while the perpendicular distance between any two diametrically opposite side walls is approximately one-fifth of an inch. The side walls are each approximately one-tenth of an inch wide. It is in such a cell, then, that the brood of the age at which it dies of sacbrood is found.

APPEARANCE OF A HEALTHY LARVA AT THE AGE AT WHICH IT DIES OF SACBROOD.

The symptoms which differentiate sacbrood from the other brood diseases are to be found primarily in the post-mortem appearances of the larvæ dead of the disease. As an aid in interpreting the description of these appearances a description of the healthy larvæ is first made.

Larvæ[1] that die of sacbrood do so almost invariably after capping and at some time during the four days just preceding the change in form of the maturing bee to that of a true pupa.

During the first two days of this prepupal period the larva moves about more or less in the cell and spins a cocoon. It is then comparatively quiet for about two days, lying on its dorsal side and ex-

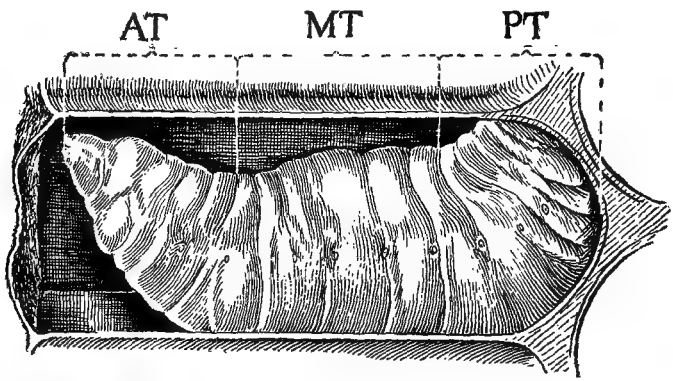


FIG. 4.—Lateral view of healthy worker larva showing the normal position within the cell. For convenience of description the length is divided into thirds—anterior third (AT), middle third (MT) and posterior third (PT). Enlarged about 8 diameters. (Original.)

tended lengthwise in the cell. At the close of this two-day period of rest, as a result of the metamorphosis going on, the larva changes very rapidly to a true pupa, assuming the outward form of an adult bee.

Although many larvæ die of sacbrood during the first two days or active period, of the 4-day prepupal period, by far the greater number of deaths occur during the last two days, the period of rest. A healthy larva at this resting period of its development is chosen, therefore, for description. As dead worker larvæ are the ones usually encountered in sacbrood and the ones almost invariably chosen in discussing the symptoms of the disease, the worker larva is here described.

The normal larva lies extended in the cell (fig. 4) on its dorsal side, motionless, and with its head pointing toward the mouth of the cell. Its posterior or caudal end lies upon the bottom of the cell,

[1] As beekeepers usually refer to the brood at this age as "larvæ," the term is used here to designate the developing bee at this stage of its growth.

while its extreme anterior or cephalic end extends almost to the cap and roof. The length of the larva is approximately one-half inch, being nearly that of the cell. Its two lateral sides cover about one-half each of the two lateral walls. The width of the larva is approximately one-fifth of an inch, being the distance between the two lateral walls of the cell.

The dorsal portion of the larva lies against the floor of the cell, being more or less convex from side to side and also from end to end. Its ventral surface is convex from side to side, and is, generally speaking, concave from end to end. Considerable empty space is found between the larva and the roof of the cell. The spiracles are visible. The glistening appearance, characteristic of a larva before capping, very largely disappears after capping. Although larvæ at this age might be thought of as white, they are in fact more or less bluish white in color. It is possible to remove a healthy larva at this age from the cell without rupturing the body wall, but care is required in doing so.

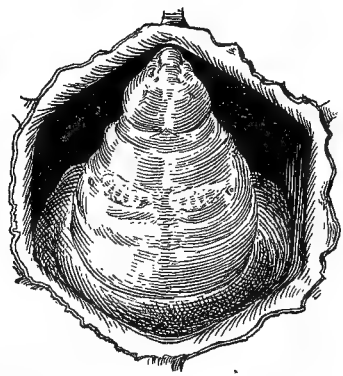


FIG. 5.—End view of healthy worker larva in normal position in the cell. Cap torn and turned aside with forceps. Enlarged about 8 diameters. (Original.)

For purposes of description it is convenient to divide the length of the larva into three parts. These may be denominated the anterior (AT), middle (MT), and posterior thirds (PT).

Anterior third.—On removing the cap from a cell the anterior cone-shaped portion of the larva is seen (fig. 5; Pl. II, *g*). The apex of this cone-shaped third is directed upward toward the angle in the roof of the cell, but is not in contact with the roof or the cap. Transverse segmental markings are to be seen. Along a portion of the median dorsal line there is frequently to be observed a narrow transparent area. A cross section of this third is circular in outline. The anterior third passes rather abruptly into the middle third. At their juncture on each lateral side, owing to a rapid increase in the width of the larva at this point, there is presented the appearance of a "shoulder."

Middle third.—This third (figs. 6 and 4; Pl. II, *m*) lies with its dorsal portion upon the floor of the cell, its axis being nearly horizontal. The ventral surface is convex from side to side, and is considerably below the roof of the cell. This upper surface is crossed from side to side by well-marked furrows and ridges representing segments of the larva. These furrows and ridges produce a deeply notched appearance at the lateral margins. In some of the segments a transverse trachea may be seen appearing as a very fine, scarcely per-

ceptible, white line. Sometimes there may be seen a narrow area along the median line of the ventral surface that is more nearly transparent than the remaining portion of the surface. This area may extend slightly into the anterior and posterior thirds. It is similar in appearance to the one on the dorsal side, but less distinct. A cross section of this third is slightly elliptical in outline. The middle third passes more or less gradually into the posterior third. The juncture on the ventral surface is indicated by a wide angle formed by the ventral surfaces of these two thirds.

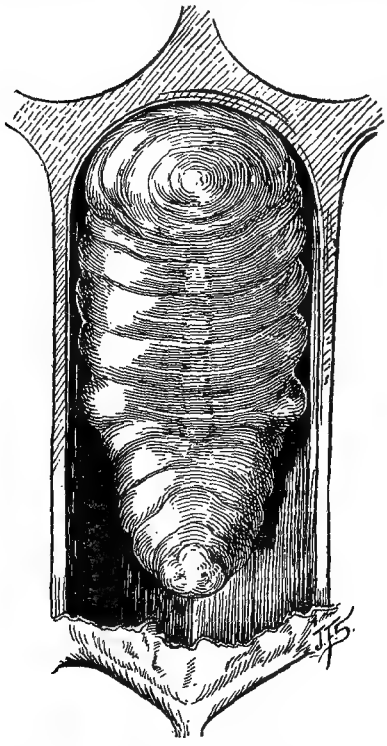


FIG. 6.—Healthy larva and cell viewed from above and at an angle. (Original.)

Posterior third.—In form the posterior third (figs. 6 and 4) is an imperfect cone, the axis of which is directed somewhat upward from the horizontal. This third occupies the bottom portion of the cavity of the cell. Its dorsal surface lies upon the bottom wall, with the extreme caudal end of the larva extending to the roof of the cell (fig. 4). The third is marked off into segments by ridges and furrows similar to, but less regular than, those of the middle third.

TISSUES OF A HEALTHY LARVA AT THE AGE AT WHICH IT DIES OF SACBROOD.

Upon removing a larva in the late larval stage and puncturing its body wall lightly, a clear fluid almost water-like in appearance flows out. This fluid consists chiefly of larval blood. By heating it, or by treating it with any one of a number of different reagents, a coagulum is formed in it. Upon rupturing the body wall sufficiently, the tissues of the larva flow out as a semiliquid mass. The more nearly solid portion of the mass appears almost white. This portion is suspended in a thin liquid, chiefly blood of the larva. A microscopic examination shows that the cellular elements of the mass are chiefly fat cells. Many fat globules suspended in the liquid tend to give it a milky appearance.

SYMPTOMS OF SACBROOD.

The condition of a colony depends naturally upon the condition of the individual bees of which it is composed. In the matter of diseases in practical apiculture the beekeeper is interested primarily in the

colony as a whole, and not in individual bees. Therefore, in describing the symptoms of a bee disease, the colony as a whole should be considered as the unit for description, and not the individual bee. A symptom of disease manifested by an individual bee, broadly considered, is, in fact, also a colony symptom. The symptoms of sacbrood as described in this paper are, therefore, those evidences of disease that are manifested by a colony affected by the disease.

It has been found that sacbrood can be produced in a healthy colony by feeding it a suspension in sirup of crushed larvæ dead of the disease. With sacbrood thus produced in experimental colonies the symptoms of the disease have been studied, and the description of these symptoms given here is based chiefly upon observations made in these experimental studies. The facts thus obtained are in accord with those observed in numerous samples of the disease sent by beekeepers from various localities in the United States for diagnosis. They are in accord, furthermore, with the symptoms as they have been observed in colonies in which the disease has appeared, not through experimental inoculation but naturally.

The symptoms of sacbrood which would ordinarily be observed through a more or less casual examination of the disease will first be considered. It must be remembered that the brood is susceptible to the disease, but that the adult bees are not.

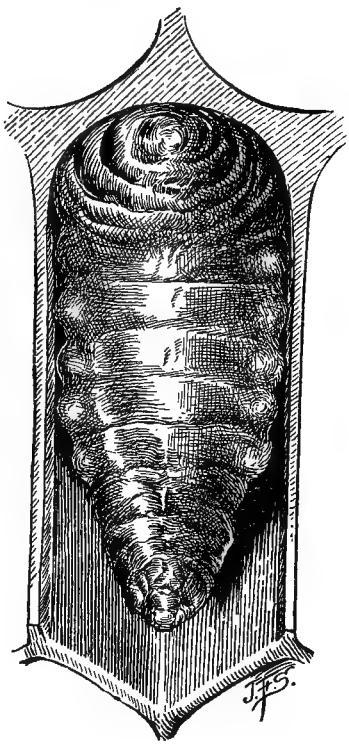


FIG. 7.—Larva dead of sacbrood lying in the cell as viewed from above and at an angle. It may have been dead a month. Cap of cell removed by bees. Enlarged about 8 diameters. (Original.)

SYMPTOMS AS OBSERVED FROM A CASUAL EXAMINATION.

The presence of dead brood is usually the first symptom observed. An irregularity in the appearance of the brood nest (Pl. I, figs. 1 and 2; Pl. IV) frequently attracts attention early in the examination. The strength of a colony in which the disease is present is often not noticeably diminished. Should a large amount of the brood become affected, however, the colony naturally becomes weakened thereby, the loss in strength soon becoming appreciable. Brood that dies of the disease does so almost invariably in capped cells, but before the pupal

stage is reached. It is rare to find a pupa dead of sacbrood (Pl. II, *zz*). The larvæ that die (fig. 7) are found lying extended lengthwise with the dorsal side on the floor of the cell. They may be found in capped (fig. 8) cells or in cells which have been uncapped (fig. 9), as bees often remove the caps from cells containing dead larvæ. Caps that are not removed are more often entire, yet not infrequently they are found to have been punctured by the bees. Usually only one puncture is found in a cap (Pl. II, *d*), but there may be two (fig. 10) or even more (Pl. II, *f*). The punctures vary in size, sometimes approximating that of a pinhead, although usually smaller, and are often irregular in outline. Sometimes a cap (fig. 11, Pl. II, *b*) has a hole through it which suggests by its position and uniform circumference that it has never been completed. Through such an opening (fig. 11; Pl. II, *e*) or through one of the larger punctures the dead larva may be seen within the cell.

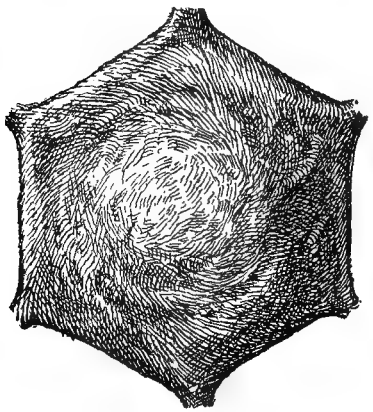


FIG. 8.—End view of capped cell which contains a larva dead of sacbrood, being similar to the one shown in figure 9. The cap here is not different from a cap of the same age over a healthy larva. (Original.)

A larva recently dead of sacbrood is slightly yellow. The color in a few days changes to brown. The shade deepens as the process of decay continues, until it appears in some instances almost black. Occasionally for a time during the process of decay the remains present a grayish appearance.

In sacbrood, during the process of decay, the body wall of the dead larva (figs. 7 and 9) toughens, permitting the easy removal of the remains intact from the cell. The content of the saclike remains, during a certain period of its decay, is watery and granular in appearance. Much of the time the form of the remains is quite similar to that of a healthy larva. If the dead larva is not removed, its surface, through evaporation of its watery content, becomes wrinkled, distorting its form. Further drying results in the formation of the

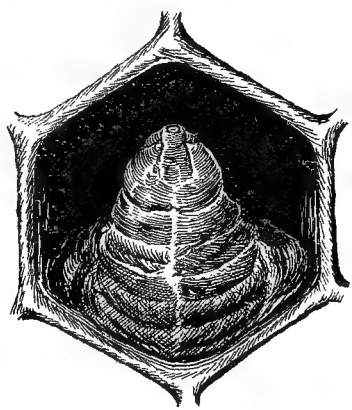


FIG. 9.—Looking into a cell containing a larva dead of sacbrood. The stage of decay is about the same as in figure 8. (Original.)

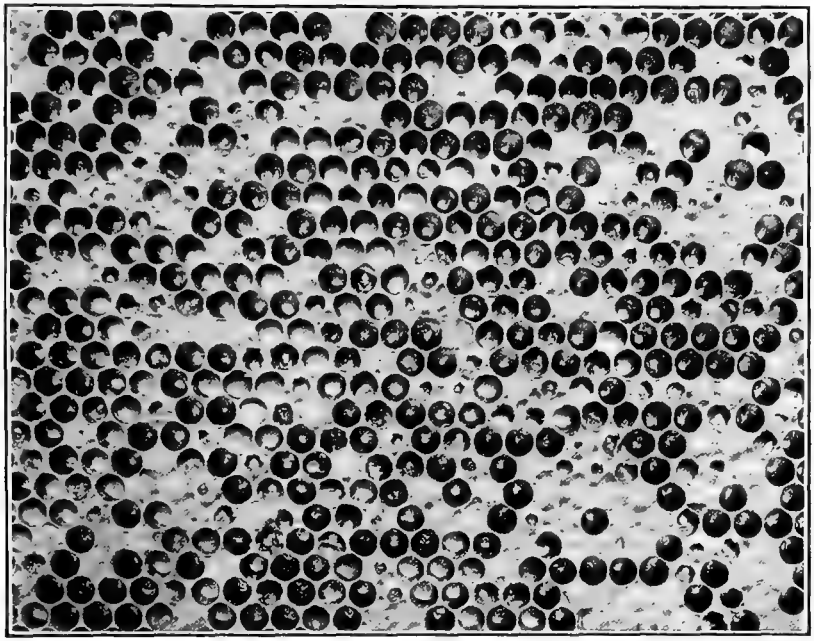


FIG. 1.—MARKED SACBROOD INFECTION. SIZE SLIGHTLY LESS THAN NATURAL. (ORIGINAL.)

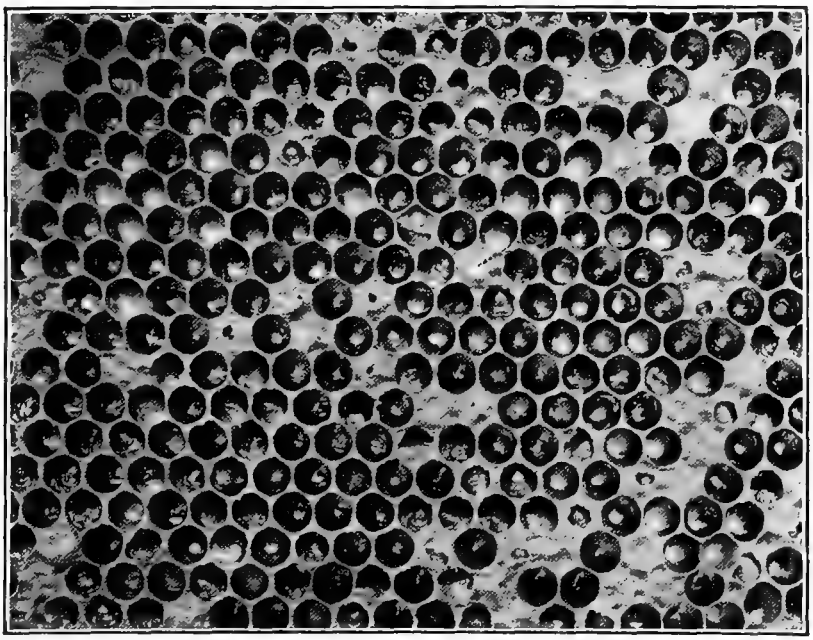
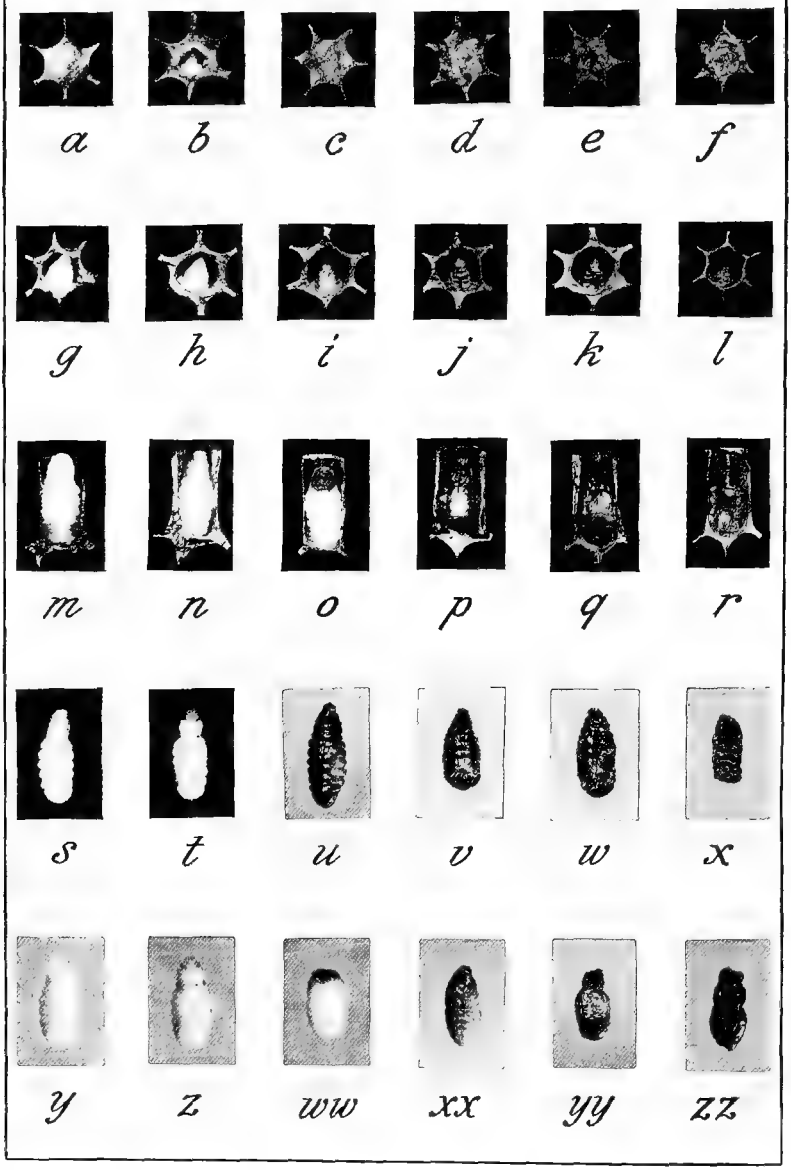


FIG. 2.—HEAVY SACBROOD INFECTION, SHOWING A NUMBER OF DIFFERENT STAGES OF DECAY OF LARVÆ. EGGS, YOUNG LARVÆ IN DIFFERENT STAGES OF DEVELOPMENT, AND DISEASED LARVÆ IN SAME AREA. NATURAL SIZE. (ORIGINAL.)

SACBROOD PRODUCED BY EXPERIMENTAL INOCULATION.



COMPARISON OF A HEALTHY LARVA AND THE REMAINS OF LARVÆ DEAD OF SACBROOD.

a, A cap of a healthy larva; *b*, *c*, *d*, *e*, and *f*, caps over larvæ in first, second, third, fourth, and fifth stages of decay, respectively; *g*, a healthy larva, end view; *h*, *i*, *j*, *k*, and *l*, an end view of the five stages of decay; *m*, a healthy larva viewed from above; *n*, *o*, *p*, *q*, and *r*, corresponding view of the five stages of decay; *s* and *y*, healthy larva removed from the cell; *t*, *u*, *v*, *w*, and *z*, larval remains in different stages of decay removed from the cell; *ww*, a larva recently dead of sacbrood with the anterior third removed by the bees; *x*, a scale removed from the cell; *xx*, larval remains from which a small portion has been removed by bees; *yy*, almost a pupa; *zz*, a pupa dead of sacbrood which had only recently transformed. (Original.)

"scale" (figs. 22, 23; Pl. II, *l*, *r*, and *x*). This scale is not adherent to the cell wall.

In sacbrood the brood combs may be said to have no odor. Larvæ undergoing later stages of decay in the disease, however, when crushed in a mass and held close to the nostrils are found to possess a disagreeable odor.

From a superficial or casual examination alone of a case of sacbrood it may be mistaken for some other abnormal condition of the brood. A careful study of the postmortem appearances of larvæ dead of the disease, however, will make it possible to avoid any such confusion. A more careful study of the dead larvæ is therefore justified.

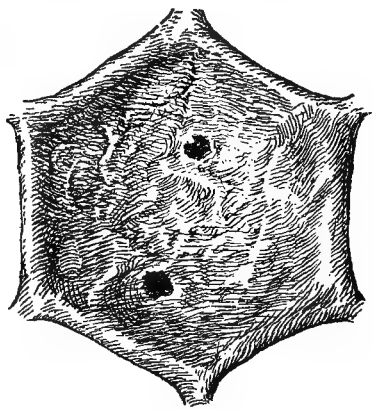


FIG. 10.—Cap of cell containing the remains of a larva dead of sacbrood. The cap is slightly sunken and bears two perforations made by the bees. (Original.)

APPEARANCE OF LARVÆ DEAD OF SACBROOD.

No signs in a larva dying of sacbrood have yet been discovered by which the exact time of death may be determined. As the larvæ in this disease usually die during the time when they are motionless, lack of movement can not be used as an early sign of death. In this description it is assumed that the larva is dead if it shows a change in color from bluish-white to yellowish or indications of a change from the normal turgidity to a condition of flaccidity.

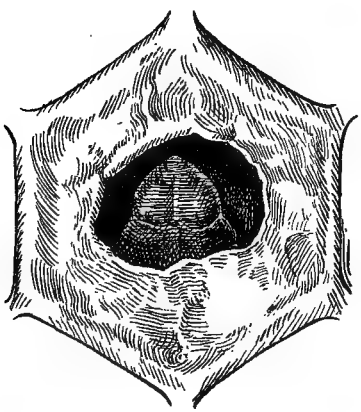


FIG. 11.—End view of cell containing a larva dead of sacbrood, with a cap which has the appearance of never having been completed. (Original.)

The appearance of a larva dead of sacbrood varies from day to day, changing gradually from that of a living healthy larva to that of the dried residue—the scale. A description that would be correct for a dead larva on one day, therefore, may and probably would be incorrect for the same larva on the following day. Moreover, all larvæ dead of the disease do not undergo the same change in appearance, causing another considerable range of variation. For convenience of description, this gradual and continual change in appearance is here considered in five more or less arbitrary stages. As the

same plan will be followed and similar terms will be used in describing these stages as were employed in the description of a healthy larva of the same age, the interpretation of the description will be aided if the appearance of a healthy larva as described above is borne in mind.

First Stage.

Uncapping a larva showing the first symptoms of the disease, it will be observed that it has assumed a slightly yellowish appearance.

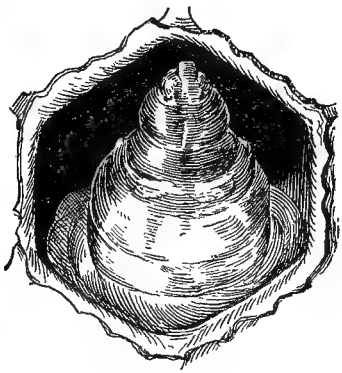


Fig. 12.—First stage: Larva showing first symptoms of sacbrood and presenting the dorsal view of the anterior third. Cap removed artificially. (Original.)

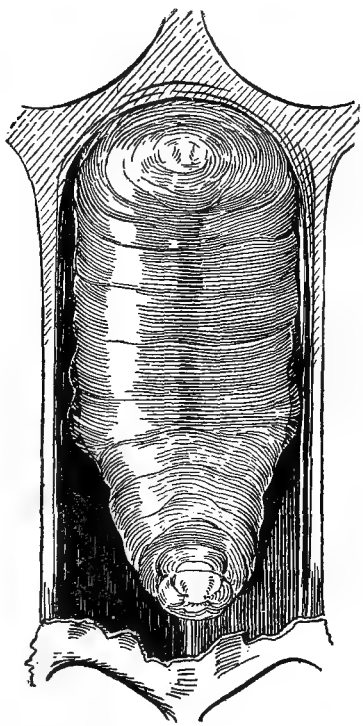


Fig. 13.—First stage: Ventral view of larva dead of sacbrood as seen from above and at an angle, giving a ventral view of all three thirds. Cap torn across. (Original.)

This shade deepens somewhat during the stage, but does not become a deep yellow.

Anterior third.—The lateral margins and extreme cephalic end of the anterior third (fig. 12; Pl. II, *b*, *h*) may have assumed, and frequently do assume, a more or less transparent appearance (represented in the figure by shading). The position and the surface markings of the anterior third are approximately those of the normal larva. When a change in the position is observed, however, the extreme anterior end of the larva—the apex of this cone-like third—having settled somewhat, does not approach so near the roof of the cell as does that of a healthy larva. It is sometimes found also that this cone-like third is deflected more or less to one side or the other.

Middle and posterior thirds.—The changes from the normal that have taken place in these two thirds are similar and can, therefore, be described together. The yellowish tint is here observed. The transverse ridges and furrows are still well marked (fig. 13). The trans-

verse tracheæ under slight magnification may be distinctly seen. The narrow, somewhat transparent area present along the ventral median line of the healthy larva is still to be seen in this stage of the decay. The lateral and posterior margins are still deeply notched and are frequently found to appear quite transparent. This appearance is due to a watery looking fluid beneath the cuticular portion of the body wall.

Sometimes only the remnant of a larva (fig. 14; Pl. II, *ww*) dead of sacbrood is found in the cell. Such remnants vary in size. The

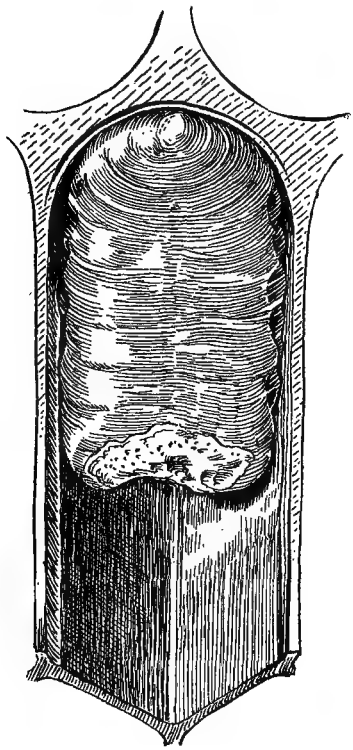


FIG. 14.—First stage: Portion of a larva dead of sacbrood, showing a more or less transverse roughened surface from which the bees have removed a portion of the larva piecemeal. (Original.)

FIG. 15.—Second stage: Dorsal view of anterior third of a larva dead of sacbrood. (Original.)

surface left from the removal of tissues is somewhat roughened, indicating that the removed portion has been taken away piecemeal, and is more or less transverse to the larva.

Consistency of the larva in the first stage.—The cuticular portion of the body wall, which chiefly constitutes the sac that characterizes the disease sacbrood, is less easily broken at this time than in the healthy larva. When the body wall is broken the tissues of the larva, which constitute the contents of the sac, flow out. This fluid tissue mass is less milky in appearance than that from a normal larva. The granular character of the contents of the sac which is marked in later stages of decay is already in evidence. By microscopic examination the granular appearance is found to be due chiefly to fat cells.

Condition of the virus in the first stage.—When larvæ of this stage are crushed, suspended in sirup, and fed to healthy bees, a large

amount of sacbrood is readily produced, showing that the larval remains in this stage are particularly infectious. This is an important fact, as it is the stage of decay at which the larva is frequently removed piecemeal from the cell.

Second Stage.

The color of the decaying larva has changed from the yellowish hue of the first stage to a brownish tint. The yellow, however, has not yet in all cases entirely disappeared.

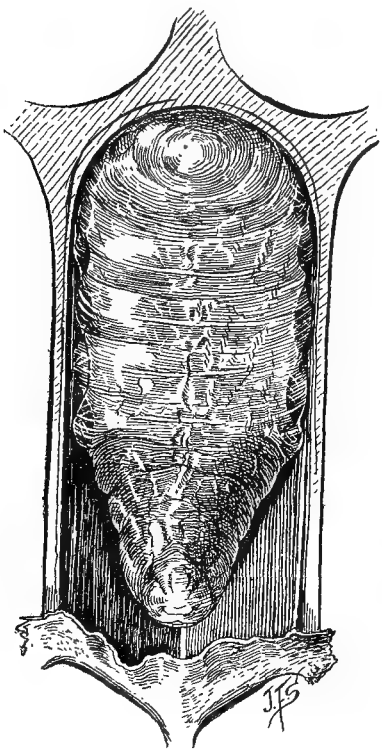


Fig. 16.—Second stage: Larva dead of sacbrood, ventral view. (Original.)

Fig. 17.—Third stage: Dorsal view of anterior third of larva dead of sacbrood. (Original.)

Anterior third.—The shade of brown is deeper in the anterior third (fig. 15; Pl. II, *i*) as a rule than in the other two thirds. On the ventral surface of the anterior third there are sometimes present minute, very dark, nearly black areas, appearing little more than mere points. Upon dissecting away the molt skin, these areas are found to be associated with the developing head and thoracic appendages of the bee. The position of the anterior third in this stage has changed only slightly from that observed in the preceding one. The apex is farther from the roof of the cell (Pl. II, *i*). The deflection is more marked and is seen in a greater number of larvæ. The surface markings have not changed materially.

Middle and posterior thirds.—The changes that have occurred in each of these two thirds are still similar and can, therefore, again be described together.

The ventral surface of these two thirds (fig. 16, Pl. II, *o*) is less convex from side to side. The ridges and furrows, representing the segments, are less pronounced. The lateral margins are still deeply notched. The prominent angle seen on the ventral side of a healthy larva, at the juncture of the middle and posterior thirds, has given place to a wider one in this stage of decay. The clear subcuticular fluid frequently observed at the lateral and posterior margins of larvæ dead of this disease is here increased in quantity.

Consistency of the contents of the sac.—The cuticular sac is now more readily observed and less easily broken. The decaying contents consist of a more or less granular-appearing mass suspended in a watery appearing fluid, the mass possessing a slightly brownish hue. The microscopic examination shows that the granular appearance is due to the presence of decaying tissue cells, chiefly fat cells, which are changing slowly as the decay of the larva goes on.

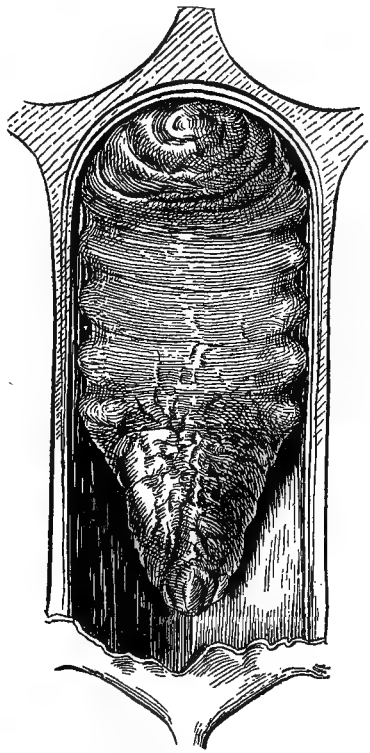


FIG. 18.—Third stage: Larva dead of sacbrood, ventral view. (Original.)

Condition of the virus.—The results of inoculations show that the remains of larvæ at this stage of decay are still in some instances infectious. The amount of infection produced when such larvæ are used in making inoculations is very much less, however, than when larvæ in the first stage are used.

THIRD STAGE.

The color of the dead larva of this stage is quite brown, that of the anterior third being a deeper shade than that of the other two thirds. An indication that the remains are drying is observed in the wrinkling of the surface that is beginning to be in evidence.

Anterior third.—The color of the anterior third is a deep brown. This third still preserves its conelike form (figs. 17 and 9; Pl. II, *j*), the distance of the apex from the roof of the cell being still further increased. This may equal one-fourth or more of the diameter of the mouth of the cell. The surface markings are still quite similar to those of a healthy larva with the exception that evidences of drying are present.

Middle third.—While the color of the middle third is similar to and often approaches in its shade that of the anterior, very frequently it is considerably lighter. The ventral surface of this third (figs. 18 and 7) is less convex from side to side than in the preceding stage, and the segmental markings, while still plainly visible, are less pronounced. The notches along the lateral margins are also less pronounced.

Posterior third.—The color of the posterior third (figs. 18 and 7; Pl. II, *p*) equals or exceeds in depth of shade that of the middle third and sometimes equals that of the anterior third. The surface markings are still pronounced and much resemble those of the normal larva.

That the watery content of the sac is being lessened through evaporation is evidenced by the diminution of the quantity of the watery-

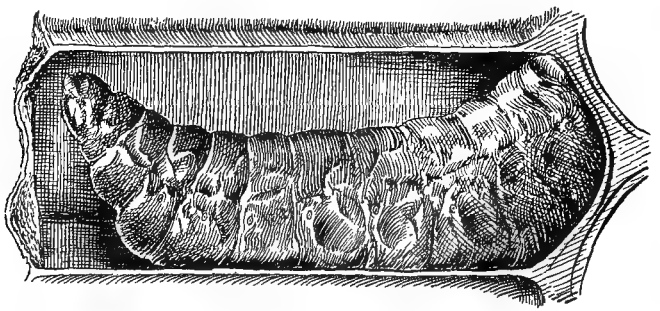


FIG. 19.—Third stage: Larva dead of sacbrood, lateral view. (Original.)

appearing substance seen at the lateral margins of the middle and posterior thirds and by the wrinkling of the cuticular sac. These wrinkles are small and numerous.

The lateral view of the larva in the third stage (fig. 19) shows that it still maintains, in a general way, the form and markings of the normal larva (fig. 4). The turgidity is gone, although the position in the cell is very much as it is in the healthy larva.

Consistency of the sac and its contents.—It is the appearance of the remains of the larva in the third stage of the decay that best characterizes the disease, sacbrood. The cuticular sac is now quite tough, permitting the removal of the larva from the cell with considerable ease and with little danger of its being torn. The content of the sac is a granular mass, brownish in color and suspended in a comparatively small quantity of a more or less clear watery-appearing fluid. Upon microscopic examination the mass is found to consist of decaying tissues, chiefly fat cells.

Condition of the virus in the third stage.—When the larval remains in this stage of decay are crushed and fed in sirup to healthy colonies no sacbrood is produced, indicating that the dead larvæ at this stage

are not infectious. The status of the virus in this stage is not definitely known, but the facts thus far obtained indicate that it is probably dead.

FOURTH STAGE.

The brown color of the larval remains has further deepened, the anterior third being much darker as a rule than the other two-thirds. The marked evidence of drying now present might be said to characterize this stage.

Anterior third.—The color is a very deep brown, often appearing almost black. As a result of drying, the apex of this conelike third

FIG. 20.—Fourth stage: Remains of larva dead of sacbrood. (Original.)

is often nearer the roof of the cell in this stage than in the preceding one. As a result it has also been drawn inward from the mouth of the cell. The surface markings seen in the normal larva are in this stage (fig. 20; Pl. II, *k*) of decay almost obliterated through the wrinkling of the surface, due to drying.

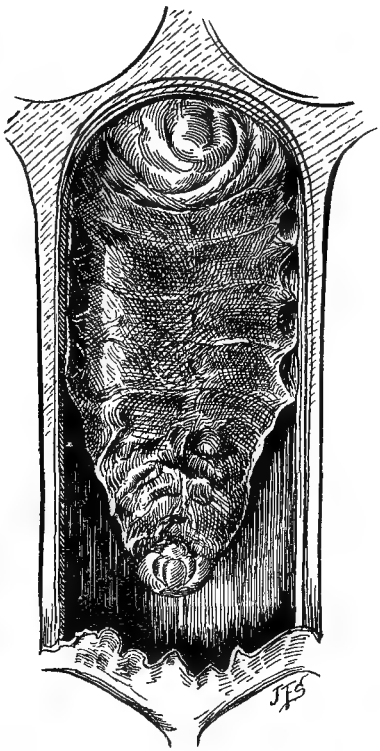


FIG. 21.—Fourth stage: Remains of larva dead of sacbrood, ventral view. (Original.)

Middle third.—This third is decidedly brown, but lighter in shade than the anterior third. The ventral surface (fig. 21; Pl. II, *q*) is slightly concave from side to side. The segmental markings are still to be seen, but are not at all prominent. The notched lateral margins extend upon the side walls of the cell. The subcuticular fluid so noticeable in some of the earlier stages has disappeared through evaporation. The effect of drying is very noticeable, causing a marked wrinkling of the surface.

Posterior third.—The posterior third (Pl. II, *q*) may or may not be darker than the middle third, but it is not darker than the anterior

third. The effect of the drying on this third is quite perceptible also. The surface markings and notched margin of the normal larva are still indicated in the decaying remains, but are much less pronounced. The subcuticular fluid is no longer in evidence.

Consistency of the contents of the sac.—Upon tearing the sac, the contents are found to be less fluid than in preceding stages. The decaying tissue mass is still granular in appearance. As the drying proceeds further the contents of the sac become pastelike in consistency.

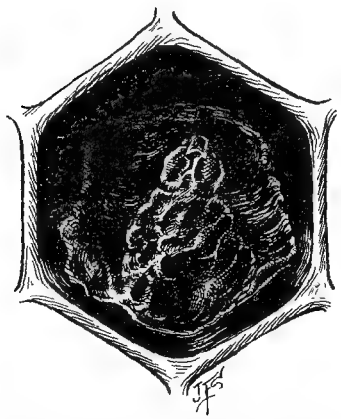


FIG. 22.—Fifth stage: Scale, or larval remains, in sacbrood as seen on looking into the cell. (Original.)

Condition of the virus in the fourth stage.—As in the preceding stage, the larval remains in the fourth stage do not seem to be infectious.

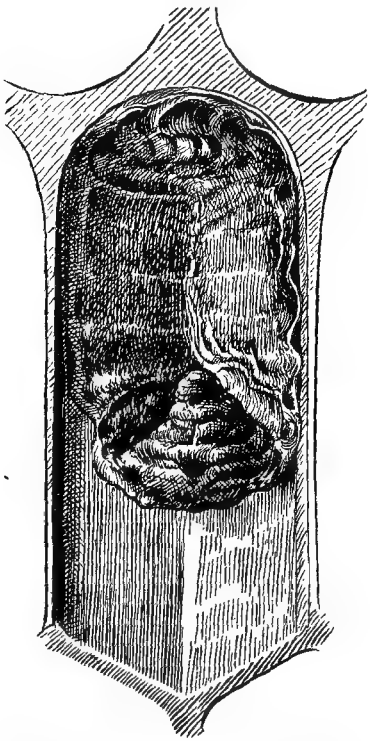


FIG. 23.—Fifth stage: Scale, or larval remains, in sacbrood viewed at an angle from above. (Original.)

FIFTH STAGE.

The dead larva in this last stage has lost by evaporation all of its moisture, leaving the dry, mummylike remains known as the "scale."

Anterior third.—The anterior third (fig. 22; Pl. II, *l*) through drying is retracted from the mouth of the cell, with the apex drawn still deeper into the cell and raised toward its roof. This third is greatly wrinkled, and, being of a very dark-brown color, presents often an almost black appearance.

Middle third.—The middle third (fig. 23; Pl. II, *r*), is deeply concave from side to side and may show remnants of the segmental markings of the larva. The surface is often roughened through drying. Sometimes both longitudinal and transverse tracheæ are

plainly visible. The margin frequently presents a wavy outline corresponding to the original furrows and ridges of the lateral margin of the larva.

Posterior third.—The posterior third (figs. 23 and 24) extends upon the bottom of the cell, but does not completely cover it. A lateral view of the scale (fig. 24) shows that it is turned upward anteriorly and drawn somewhat toward the bottom of the cell. The ventral surface is concave, often roughened, and directed somewhat forward. This margin, like that of the middle third, has a tendency toward being irregular.

The scale.—The scale can easily be removed intact from the cell. (Pl. II, *x*.) Indeed, when very dry, many of them can be shaken from the brood comb. When out of the cell, they vary markedly in appearance. The anterior third is of a deeper brown than the the other two thirds as a rule. The dorsal side of the middle and

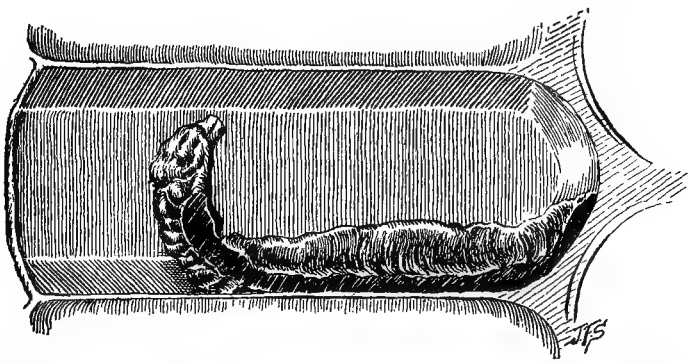


FIG. 24.—Scale, or larval remains, in position in cell cut lengthwise, lateral view. (Original.)

posterior thirds is shaped to conform to the floor of the cell, being in general convex, with a surface that is smooth and polished. The margin is thin and wavy. The anterior third and the lateral sides of the middle and posterior thirds being turned upward, the ventral surface being concave, and the posterior side being convex, the scale in general presents a boatlike appearance and could be styled "gondola-shaped." This general form of the scale has been referred to by beekeepers as being that of a Chinaman's shoe. When completely dry, the scale is brittle and may easily be ground to a powder.

Condition of the virus in the scale.—The scales in sacbrood, when fed to healthy bees, have shown no evidence of being infectious.

The length of time that dead larvæ are permitted by the bees to remain in the cells before they are removed varies. They may be removed soon after death, they may remain until or after they have become a dry scale, or they may be removed at any intervening stage in their decay. Not infrequently they are permitted to remain to or

through the stage described above as the third stage (figs. 7, 9, 17, and 18; Pl. II *j*, *p*). That the dead larvæ are allowed to remain in the cells often for weeks is in part the cause of the irregularity observed in the appearance of the brood combs (p. 11). (Pls. I, IV.)

APPEARANCE OF THE TISSUES OF A LARVA DEAD OF SACBROOD.

The gross appearance of a larva during its decay after death from sacbrood has just been described. The saclike appearance of the remains, with its subcuticular watery-like fluid and its granular content, can better be interpreted by knowing something of the microscopic structure of the dead larva.

A section through a larva (fig. 25, A) dead of sacbrood shows that the fat tissue constitutes the greater portion of the bulk of the body. The fat cells (FC) are comparatively large. In the prepared section when considerably magnified (C) they are seen to be irregular in outline, with an irregular-shaped nucleus (Nu). Bodies stained black, more or less spherical in form and varying in size, are found in them. The presence of these cells is the chief cause for the granular appearance of the contents of larvæ dead of sacbrood. This appearance has often been observed by beekeepers and is a well-recognized symptom of sacbrood.

In the section (A) may be seen a molt skin (C_2), which is at a considerable distance from the hypodermis (Hyp). Another cuticula (C_1) is already quite well formed and lies near the hypodermis. Between these two cuticulæ (C_2 and C_1) during the earlier stages of decay there is a considerable space ("intercuticular space") (IS). This space is filled with a watery-looking fluid. That the fluid is not water, but that it is of such a nature that a coagulum is formed in it during the preparation of the tissues for study, is shown by the presence of a coagulum in the sections.

The body (B, A) wall of the larva is composed of the cuticula (C_1), the hypodermis (Hyp) and the basement membrane (BM). The hypodermal cells may be present in the mass content of the larval remains. These cells are comparatively small. Similar ones are to be found in the tracheal walls (Tra). These cells, however, make up only a small portion of the contents of the sac.

There are many other cellular elements to be found in the decaying mass of larval tissues, some of which contribute to this granular appearance. Among these are the œnocytes (Oe), cells (D) larger than the fat cells, but comparatively few in number. These are found among the fat cells, especially in the ventral half of the body. The œnocytes in the prepared tissues are irregular in outline, having a nucleus regular in outline. The cytoplasm is uniformly granular and does not contain the black staining bodies found in the fat cells (C).

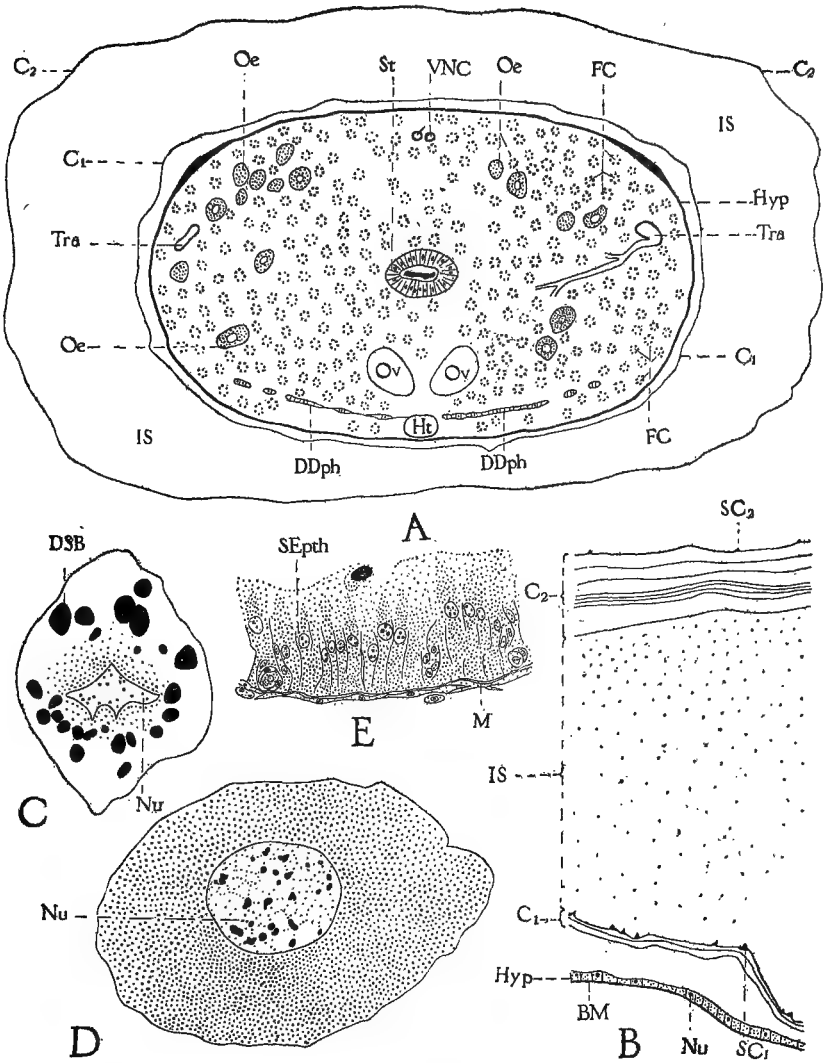


FIG. 25.—The tissues of a worker larva after being dead of sacbrood about one week. A, cross section, semidiagrammatic, of the abdomen in the region of the ovaries, showing a recently cast cuticula, or *molt skin* (C_2), a *newly formed cuticula* (C_1), the *hypodermis* (Hyp), the *stomach* (St), the *ovaries* (Ov), the heart (Ht), the ventral nerve cord (VNC), the dorsal diaphragm (DDph), tracheæ (Tra), œnocytes (Oe), and fat cells (FC). Between the cuticula C_2 and the cuticula C_1 is a considerable intercuticular space (IS). B represents the body wall in this pathological condition, showing the cuticula C_2 and the cuticula C_1, both bearing spines (SC_2 and SC_1), and the intercuticular space (IS) in which is found evidence of a coagulum formed from the fluid filling the space by the action of the fixing fluids. The remainder of the body wall, the hypodermis (Hyp), and the basement membrane (BM) are also shown. C, fat cell with irregular outline, irregular nucleus (Nu), and deep staining bodies (DSB). D, œnocyte with uniformly staining cytoplasm, and with a nucleus (Nu) having a uniform outline. E, a portion of the stomach wall showing the epithelium (SEpth) during metamorphosis, it being at this time quite columnar in type, and the musculature (M). (Original.)

The molt skin (C_2) is probably the one that is shed normally about three days after the larva is capped. The cuticula (C_1), already quite well formed, is probably the one which normally would have entered into the formation of the molt skin that is cast at the time the larva or semipupa changes to a pupa. The molt skin (C_2) constitutes for the most part the sac which is seen to inclose the decaying larval mass in sacbrood, the cuticula (C_1) probably assisting somewhat at times. The presence of the subcuticular fluid is made more intelligible by these facts. Larvæ dying of sacbrood at an earlier or later period in their development will present an appearance varying somewhat from that just described.

Contrasted with the stomach (midintestine or midgut) of a feeding larva, the stomach (A, St) of a larva at the age at which it dies of sacbrood is small. The cells lining the wall of the organ vary considerably in size and shape, depending upon the exact time at which death takes place. In contrast to the low cells of the stomach wall in younger larvæ, the cells (E, SEpth) at this later period are much elongated. These cells would also at times be found in the decaying granular mass present in the larval remains.

The various organs of the body contribute to the cellular content of the decaying larval mass. At the period at which the larva dies of sacbrood, the cellular changes accompanying metamorphosis are particularly marked. This condition introduces various cellular elements into the decaying larval mass.

The granular mass from the larval remains in sacbrood is, therefore, a composite affair. Upon examining the mass microscopically, it will be found that the granular appearance is due for the most part to fat cells suspended in a liquid. The liquid portion seems to be chiefly blood of the larva, or, at least, derived from the blood, although augmented most probably by other liquids of the larva and possibly by a liquefaction of some of the tissues present. The granular mass suspended in a watery fluid, as a symptom of sacbrood, is by these facts rendered more easily understood.

CAUSE OF SACBROOD.

Doolittle (1881), Jones (1883), Simmins (1887), Root (1892 and 1896), Cook (1902), Dadant (1906), and others through their writings have pointed out the fact that there are losses sustained from sacbrood. There has been no consensus of opinion, however, as to the infectiousness of the disease. On this point Dadant (1906) writes:

> Whatever may be the cause of this disease (so-called Pickled Brood), and although it is to a certain extent contagious, it often passes off without treatment. But, as colonies may be entirely ruined by it, it ought not to be neglected.

In the quotation Dadant expresses the belief that the disease is an infectious one. This view has been proved by recent studies to be the correct one. Since the disease is one of a somewhat transient nature, often subsiding and disappearing quickly without treatment, and is quite different in many ways from the foulbroods, it is not strange that some writers should have held that it is not infectious.

PREDISPOSING CAUSES.

Beekeepers have known for many years certain facts concerning the predisposing causes of sacbrood. Recent studies have added others relative to sex, age, race, climatic conditions, season, and food as possible predisposing factors in the causation of the disease.

Age.—The results of the studies suggest that adult bees are not directly susceptible to the disease. Pupæ are rarely affected (Pl. II, *zz*). If one succumbs to the disease, it is quite soon after transformation from the larval stage. Primarily it is the larvæ that are susceptible. When a larva dies of the disease, it does so almost invariably after capping, and usually during the 2-day period immediately preceding the time for the change to a pupa.

Sex.—Worker and drone larvæ may become infected. Queen larvæ apparently are also susceptible, although this point has not yet been completely demonstrated.

Race.—No complete immunity against sacbrood has yet been found to exist in any race of bees commonly kept in America. That one race is less susceptible to the disease than another may be said to be probable, although the extent of such immunity has not been established.

The question: "What race of bees is there in the diseased colony?" was asked beekeepers sending samples of diseased brood. Out of 140 replies received from those sending sacbrood samples, 53 reported hybrids, 49 reported Italians, 21 reported blacks, and 17 reported Italian hybrids. These replies show that the bees commonly kept by American beekeepers are susceptible, although their relative susceptibility is not shown.

The bees which have been inoculated in the experimental work on sacbrood have been largely Italians or mixed with Italian blood. Blacks have also been used. No complete immunity was observed in any colony inoculated. That the blacks are more susceptible than strains having Italian blood in them is suggested by some of the results. Facts concerning the problem of immunity as relating to bees are yet altogether too meager to justify more definite statements.

Climate.—Historial evidence strongly suggests that sacbrood is found in Germany (Langstroth, 1857), England (Simmins, 1887),

and Switzerland (Burri, 1906). Beuhne (1913) reports its presence in Australia, and Bahr (1915) has encountered a brood disorder among bees in Denmark which he finds is neither of the foul broods. He had examined 10 samples of it but had not studied it further. He says it may be sacbrood.

About 400 cases of sacbrood have been diagnosed by Dr. A. H. McCray and the writer among the samples of brood received for examination at the Bureau of Entomology. A few of these were obtained from Canada. Whether the disease occurs in tropical climates or the coldest climates in which bees are kept has not yet been completely established.

The mountains and coast plain of the eastern United States, the plains of the Mississippi Valley and the mountains, plateaus, and coast plain of the western portion of the country have contributed to the number of samples examined. It occurs in the South and the North.

Its occurrence in such widely different localities is proof that sacbrood is of such a nature that it can appear under widely different climatic conditions. The relative frequency of the disease, furthermore, is not materially different in the different sections of the country. It must be said, however, that the extent, if any, to which the disease is affected by climate has not yet been determined.

The practical import of these observations regarding climate, of particular interest here, is that the presence of sacbrood in any region can not be attributed entirely to the prevailing climatic conditions.

Season.—It has long been known that sacbrood appears most often and in the greatest severity during the spring of the year. As is shown by the results obtained in the diagnosis of it in the laboratory, the disease may appear at any season of the year at which brood is being reared. In the inoculation experiments sacbrood has been produced with ease from early spring to October 21. While it is thus shown that the brood is susceptible to sacbrood at all seasons, various factors together cause the disease to occur with greater frequency during the spring.

Food.—Before it was known that sacbrood is an infectious disease the quantity or quality of food was not infrequently mentioned by beekeepers as being the cause of the disease. Since a filterable virus has been shown to be the exciting cause of the disease, it is left to be considered whether food is a predisposing cause. The distribution of the disease mentioned above, under the heading "Climate," here again serves a useful purpose. Since it occurs in such a wide range of localities, wherein the food and water used by the bees vary as greatly almost as is possible in the United States, the conclusion may be drawn that its occurrence is not dependent upon food of any restricted character. Furthermore, sacbrood is found in colonies having an abundant supply of food, as well as in colonies having a

scarcity. It has been produced experimentally in colonies under equally varying conditions in regard to the quantity of food.

While it is possible that the quantity or quality of food may influence somewhat the course of the disease in the colony, the rôle played by food in the causation of sacbrood must be slight, if indeed it contributes at all appreciably to it. Practically, therefore, for the present it may be considered that neither the quality nor quantity of food predisposes to this disease.

EXCITING CAUSE OF SACBROOD.

That sacbrood is an infectious disease was demonstrated by the writer (1913) through experiments performed during the summer of 1912. This was done by feeding to healthy colonies the crushed tissues of larvæ dead of sacbrood, suspended in sugar sirup. The experiments were performed under various conditions, and it was found that the disease could be produced at will, demonstrating thereby that it was actually an infectious one.

In the crushed larval mass no microorganisms were found either microscopically or culturally to which the infection could be attributed, although the experiments had proved that the larva dead of the disease did contain the infecting agent. This led to the next step in the investigation, which was to determine whether the virus was so small that it had not been observed, and whether its nature would permit its passage through a filter. The first filter used for this purpose was the Berkefeld.

The process by which the filtration is done is briefly this: Larvæ which have been dead of sacbrood only a few days are picked from the brood comb and crushed. The crushed mass is added to water in the proportion of 1 part larval mass to 10 parts water. A higher dilution may be used. This aqueous suspension is allowed to stand for some hours, preferably overnight. To remove the fragments of the larval tissues still remaining, the suspension is filtered, using filter paper. The filtrate thus obtained is then filtered by the use of the Berkefeld filter [1] (fig. 26) properly prepared. The filtering in the case of the coarser filters especially can be done through gravity alone.

To determine whether any visible microorganisms are present in this last filtrate, it is examined microscopically and culturally. When found to be apparently free from such microorganisms, a quantity of it may be added to sirup and the mixture fed to healthy colo-

[1] The Berkefeld filter consists of a compact material (infusorial earth) in the form of a cylinder. A glass mantel (A) in which is fixed the filter forms a cup for holding the fluid to be filtered. Having filtered the aqueous suspension of crushed sacbrood larvæ through paper, the filtrate is then filtered by allowing it to pass through the walls of the Berkefeld cylinder (B). The filtrate from this filtration is collected into a sterile flask (F) through a glass tube (D) with its rubber connection (C). In filtering in this instance gravity is the only force used.

nies. When all this is properly done, sacbrood will appear in the inoculated colonies. This shows that the virus [1] of this disease, to a

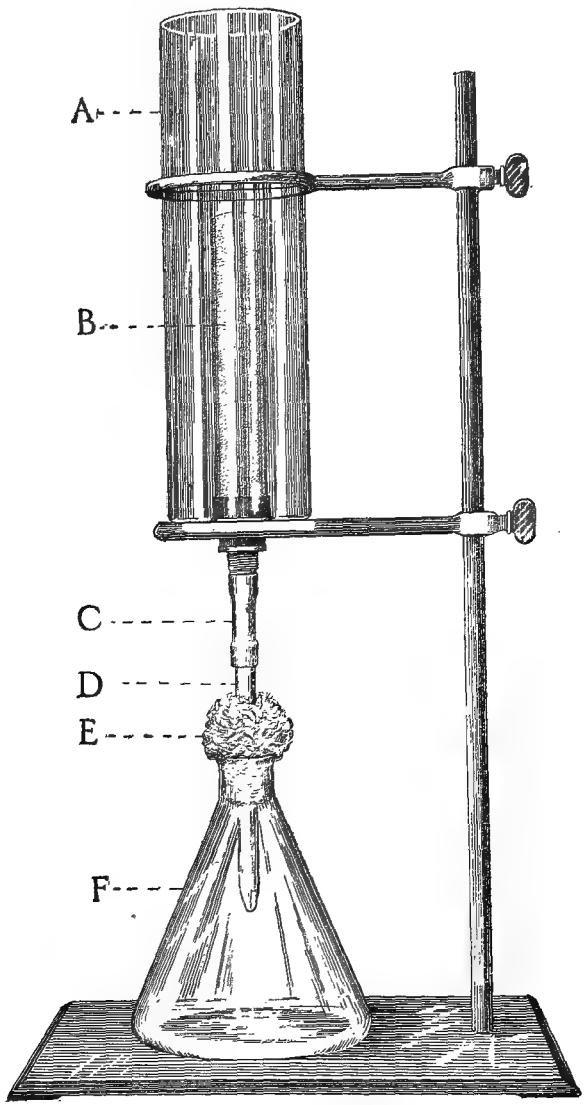


FIG. 26.—Berkefeld filter (B) with the glass mantle (A), glass tubing (D), a connecting rubber tubing (C), and a flask (F) with a cotton plug (E). (Original.)

certain extent, at least, passes through the Berkefeld filter. With this filter the virus is therefore filterable.

[1] In referring to the infecting agent in sacbrood, the term "virus" is preferable to the terms "germ" or "parasite." In relation to the disease, however, its meaning is the same as that conveyed by the latter terms.

In the study of the virus of sacbrood use has been made also of the Pasteur-Chamberland filter [1] (fig. 27). This is a clay filter, the pores of which are much finer than those of the Berkefeld used. In using this filter, an aqueous suspension of larvæ dead of the disease is prepared as before. This is filtered by the aid of pressure obtained

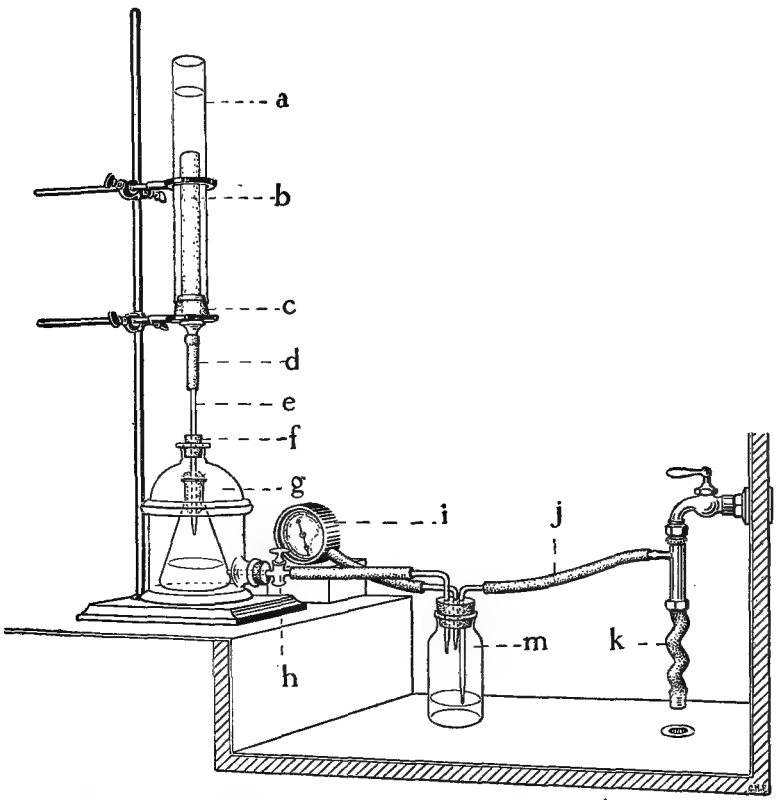


FIG. 27.—A convenient apparatus which can be employed in using the Pasteur-Chamberland, Berkefeld, and other filters. Pasteur-Chamberland filter (b) with a glass mantle (a), a rubber stopper (c) through which passes the filter, a connecting rubber tubing (d), glass tubing (e), a perforated rubber stopper (f), a vacuum jar (g), designed by the writer, in which is placed a cotton-stoppered and sterilized flask, a glass stopcock (h), a vacuum gauge (i), a reservoir (m) with pressure-rubber connections (j), and a vacuum pump (k). (Original.)

by means of a partial vacuum in an apparatus devised for this purpose. Filtrates obtained from this filter when fed to healthy colonies produced the disease. Since the virus of sacbrood will pass through

[1] The Pasteur-Chamberland filter consists of clay molded in the form of a hollow cylinder and baked. This is used with a glass cylinder (a) fitted with a rubber stopper (c). In the use of this filter, force is employed. This was obtained for these experiments through the use of a jar (g) devised by the writer in which a partial vacuum can be produced. In this jar, is placed a flask plugged with cotton and sterilized. Connections are made as shown in the illustration, the vacuum being produced through the use of the pump (k). In less than half an hour usually a half-pint of filtrate can be obtained with this apparatus.

the pores of the Pasteur-Chamberland filter also, it is therefore filterable and is very properly referred to as a "filterable"[1] virus.

In considering the virus of sacbrood it is suggested that the beekeeper think of it as a microorganism [2] which is so small or of such a nature that it has not been seen, and which will pass through the pores of fine clay filters. This conception of it will at least make it more easily understood.

WEAKENING EFFECT OF SACBROOD UPON A COLONY.

The first inoculations in proving that sacbrood is an infectious disease were made on June 25, 1912. Two colonies were used, each being fed with material from a different source. The inoculation feedings were made on successive days. Sacbrood having been produced in the colonies, the inoculations were continued at intervals throughout July and August. During this period, a large amount of sacbrood was present in both colonies. By the end of July these colonies had become noticeably weakened, and by the end of August they had become very much weakened, as a result of the sacbrood present in them. On September 5 one of the colonies swarmed out.

The brood (Pl. IV) of this colony, large in quantity, was practically all dying of sacbrood. The other colony, when examined on September 16, was found to be very weak. At this time, however, most of the dead brood had been removed and healthy brood was being reared. This colony increased in strength and wintered successfully.

The results obtained from the inoculation of these two colonies demonstrated not only that sacbrood is an infectious disease, but also that the disease in a colony tends to weaken it. The results indicate also that a colony may be destroyed by the disease, or it may recover from it, gain in strength, and winter successfully.

Each year since 1912 two or more colonies have been fed sacbrood material at intervals during the brood-rearing season for the purpose of obtaining disease material for experimental purposes. The inoculated colonies in all instances have shown a tendency to become weakened as a result of the inoculations.

The death of the worker larvæ is the primary cause for the weakness resulting from the disease in a colony. Another point to be thought of is that dead sacbrood larvæ remaining in the cells for weeks, as they not infrequently do, reduce the capacity of the brood nest for brood rearing, which has a tendency also to weaken the colony.

[1] In searching the tissues of larvæ dead of sacbrood and the filtrates obtained from them nothing has been discovered by the aid of the microscope, or culturally, which has yet been demonstrated as being the infecting agent. This being true, the virus could be spoken of tentatively as an "ultramicroscopic virus." It is preferable, for the present, however, to refer to it simply as a filterable virus.

[2] There is some question whether, in the case of diseases having a virus which is filterable, the infecting agent is in every instance a microorganism. The evidence is strong, however, that it is.

AMOUNT OF VIRUS REQUIRED TO PRODUCE THE DISEASE, AND THE RAPIDITY OF ITS INCREASE.

Assuming the virus of sacbrood to be a very minute microorganism, the number of germs present in a larva dying of the disease must be considered as exceedingly large. Whether a single germ taken up by a larva will produce the disease in every instance, or in any instance, is not known. If the disease does result at any time from the ingestion of a single germ, all of the conditions, it may be assumed, must be especially favorable for the production of the disease. From what is known of diseases of other animals and of man, and from the results thus far obtained in the study of sacbrood, it is well, at present, to assume that the number of sacbrood germs taken up by a larva may be so small that no disease results.

It is certain, however, that a comparatively small number of sacbrood germs ingested by a larva about two days old are sufficient to produce the disease. That the few germs thus taken up can increase within the larva during an incubation period of five or six days to such a vast number as is assumed to be present in a larva dying of the disease indicates the extreme rapidity with which the germs are able to multiply.

The minimum quantity of virus necessary to produce a moderate infection in a colony has not been definitely determined. It was found by experiments, however, that the virus contained in a single larva recently dead of the disease was sufficient to produce a large amount of sacbrood in a colony.

As a very rough estimate, it may be said that the quantity of virus in a single larva dead of sacbrood is sufficient, when suspended in half a pint of sirup and fed to a healthy colony, to produce infection in and death of at least 3,000 larvæ. Starting then with the virus contained in a single larva, in less than one week it would easily be possible to have 3,000 larvæ dead of the disease, which means that the virus has been increased 3,000-fold within one week. This latter amount of virus would be sufficient to produce an equal amount of infection in 3,000 colonies, increasing the amount of virus again 3,000-fold. In less than two weeks, therefore, theoretically it would be possible to produce a sufficient amount of virus to infect 9,000,000 colonies, more colonies probably than are to be found at present in the United States. Carrying the idea somewhat further, within three weeks, theoretically enough virus could be produced to inoculate every colony in existence.

These facts are sufficient to indicate somewhat the enormous rapidity with which the virus of sacbrood is capable of increasing.

METHODS USED IN MAKING EXPERIMENTAL INOCULATIONS.

The laboratory study of bee diseases being new, it has been necessary in many instances to devise new methods. In the experimental inoculations of bees the methods used have undergone revision from time to time. Those now employed have proved quite satisfactory.

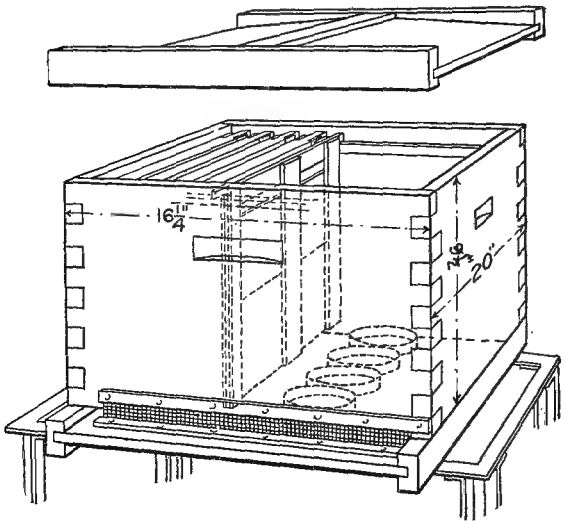


FIG. 28.—The hive as it is employed to house and feed a colony used for experimental inoculations. Here are shown four Hoffman frames, a division board, four open Petri dishes as feeders, and the entrance nearly closed with wire cloth, the opening being on the side of the hive body occupied by the colony. The dimensions indicated are approximate. The angle at which the hive was photographed for this drawing caused its length to appear foreshortened. (Original.)

As the virus of sacbrood has not been cultivated in the laboratory artificially, it has been necessary in these investigations to inoculate a large number of colonies. A nucleus of bees that could be accommodated on from 3 to 6 brood frames was found to serve very satisfactorily the purpose of an experimental colony. The queen should always be clipped. The frames are placed in one side of a 10-frame hive body (fig. 28). Over the entrance to the hive is placed wire cloth, leaving a small space of about 1 inch in length on the side occupied by the brood frames. Petri dishes[1] (fig. 29) serve well the purpose of a feeder. Both halves of the dish are used as receptacles. These are placed, preferably about four of the halves, within the hive on the bottom board on the side not occupied by frames. The hives of the experimental apiary (Pl. III) are arranged chiefly in pairs, with the entrances of consecutive rows pointing in opposite directions. The space occupied by the apiary should be

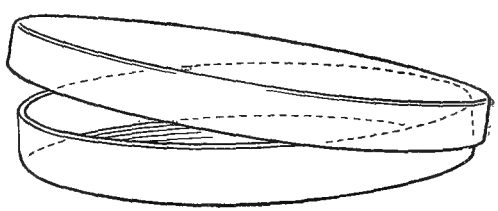


FIG. 29.—Petri dish. The top half is slightly raised. Those used here are 4 inches in diameter. (Original.)

[1] A Petri dish, a much-used piece of apparatus in a laboratory, is simply a shallow, circular, glass dish with a flat bottom and perpendicular sides. It consists of two halves, a bottom and a top. These are very similar. The top half, being slightly larger, fits over the bottom one when the two halves are placed together.

A View of the Experimental Apiary of 54 Colonies in which the Inoculation Experiments made During the Summer of 1915 were Conducted. (Original.)

BROOD FRAME CONTAINING SACBROOD, TAKEN FROM AN EXPERIMENTAL COLONY IN WHICH THE DISEASE WAS PRODUCED BY FEEDING THE VIRUS OF THE DISEASE. (ORIGINAL.)

broken up, preferably by trees or shrubbery. By these means, it will be observed, there is a tendency to minimize the likelihood of robbing, swarming, absconding, and accidental straying or drifting of bees to foreign colonies.

In preparing the material with which the colony is inoculated, larvæ in early stages of the disease are picked from the brood frames, crushed, and added to sugar sirup. The crushed mass from 10 or more sacbrood larvæ, suspended in somewhat more than half a pint of sugar sirup, has been found to be a suitable quantity of the infective material to use in making an inoculation. The suspension may be fed to the bees as one feeding or more. The inoculation feedings should be made as a rule toward evening to avoid the tendency to rob, which may be noticed during a dearth of nectar. Inoculations should not be made when the tendency to rob is at all marked.

FIG. 30.—Capillary pipette. A piece of glass tubing drawn to capillary size at one end. Reduced to three-fourths of the size used. (Original.)

Before a colony is inoculated it should be determined that its activities are normal. A colony should not be inoculated for several days after it has been made by division, or immediately after its removal from a foreign location. An experimental colony when inoculated should have larvæ of all ages, and a queen doing well.

Between five and six days after a colony has been inoculated with sacbrood virus, the first symptoms of the disease are to be expected. The finding of capped larvæ having a slightly yellowish hue (fig. 12; Pl. II, *b*, *h*) is the best early symptom by which the presence of the disease may be known.

Another method of inoculation may be used and under certain circumstances is desirable. The method is more direct than the one just described. The crushed tissues of a diseased larva are suspended in a small amount of water or thin sugar sirup. With a capillary pipette (fig. 30) made from small glass tubing, a very small amount of the suspension is added directly to the food which surrounds the healthy larva in the cell. This is easily done. Having drawn some of the suspension into the pipette, carefully touch the food in the cell surrounding the larva with the point of the pipette. A small amount of the suspension will flow out and mix with the food. Larvæ approximately two days of age should be selected for feeding. A dozen

or more should be fed in making an inoculation. The area of brood inoculated may be designated by marking on the brood frame, or by removing the brood from around the area inoculated, thus marking it off.

MEANS FOR THE DESTRUCTION OF THE VIRUS OF SACBROOD.

Although the virus of sacbrood may increase with great rapidity, fortunately it is quite as readily destroyed. Nature supplies many means by which this may be accomplished. While theoretically a sufficient amount of virus may be produced within one month to inoculate all the bees in existence, within another month, if left to natural means alone, practically all such virus would be destroyed. This latter fact constitutes one of the chief reasons for the comparatively rapid self-recovery of colonies from this disease.

It was observed in the experiments that larvæ dead of sacbrood when left in the brood comb ceased to be infectious in less than one month after death.

HEATING REQUIRED TO DESTROY SACBROOD VIRUS WHEN SUSPENDED IN WATER.

Approximate results have been published (White, 1914) relative to the heating that is necessary to destroy the virus of sacbrood when it is suspended in water. In the following table are given some results which have been obtained:

TABLE I.—*Effect of heating on the virus of sacbrood suspended in water.*[1]

Date of inoculation.	Temperature.		Time of heating.	Results of inoculation.
	°F.	°C.	*Minutes.*	
Aug. 6, 1913	122	50	30	Sacbrood produced.
Sept. 10, 1913	131	55	10	Do.
Sept. 9, 1913	131	55	20	Do.
Sept. 18, 1913	135	57	15	Do.
June 30, 1915	136	58	10	Do.
Sept. 10, 1913	136	58	10	No disease produced.
Aug. 28, 1915	138	59	10	Do.
Sept. 10, 1913	140	60	15	Do.
Aug. 28, 1915	142	61	10	Do.
Aug. 26, 1913	149	65	15	Do.
Do	158	70	15	Do.
Do	167	75	15	Do.
Do	176	80	15	Do.

[1] Fractions will be omitted in this paper, the nearest whole number being given.

It will be observed from Table I that 138° F. (59° C.) maintained for 10 minutes was sufficient to destroy the virus of sacbrood in the inoculation experiments recorded. Technically, in view of the variable factors which must be considered in experiments of this kind, this result, as representing the thermal death point of the sacbrood virus, should be considered as being only approximate. For practical purposes, however, it is sufficient.

In performing these experiments a crushed mass, representing from 10 to 20 larvæ recently dead of the disease, is diluted to about 10 times its volume with tap water. About one-half ounce of this suspension is placed in a test tube (fig. 31), almost filling it. The tube is stoppered with a perforated cork, bearing a short glass tube of small caliber and drawn at one end to capillary size. This is all immersed in water at a temperature to which it is desired that the virus shall be heated. It requires nearly five minutes for the temperature of the suspension in the tube to reach that of the water outside. After reaching the degree desired the temperature is maintained for 10 minutes, after which the tube is removed and the contents added to about one-half pint of sirup. The suspension is then fed to a healthy colony. If by such a feeding no sacbrood is produced, the virus is considered as having been destroyed by the heating. On the other hand, if the disease is produced it follows naturally that the virus had not been destroyed.

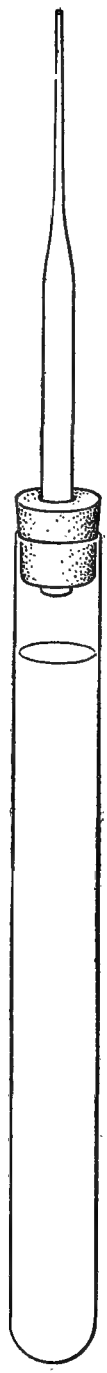


FIG. 31.—Tube in which a suspension of sacbrood larvæ is placed for heating. It consists of a test tube one-half inch in diameter supplied with a perforated stopper through which passes a short piece of glass tubing drawn at one end to capillary size. (Original.)

HEATING REQUIRED TO DESTROY SACBROOD VIRUS WHEN SUSPENDED IN GLYCERINE.

In determining the amount of heating that is necessary to destroy the virus of a disease when it is suspended in a liquid, the results should always be given in terms of at least the three factors, (1) degree of temperature, (2) time of heating, and (3) the medium in which the virus is suspended.

With the virus of sacbrood the results vary markedly, depending upon the nature of the liquid in which the suspension is made. To illustrate this point the results of a few inoculation experiments are given here in which the virus was heated while suspended in glycerine.

TABLE II.—*Effect produced by heating the virus of sacbrood suspended in glycerine.*

Date of inoculation.	Temperature.		Time of heating.	Results of inoculation.
	°F.	°C.	*Minutes.*	
June 25, 1915	140	60	10	Sacbrood produced.
June 24, 1915	149	65	10	Do.
June 25, 1915	158	70	10	Do.
Aug. 28, 1915	160	71	10	Do.
Do	163	73	10	No disease produced.
Aug. 7, 1915	167	75	10	Do.

In these inoculations it will be observed that a temperature somewhat greater than 158° F. (70° C.) maintained for 10 minutes was necessary to destroy the virus of sacbrood when it was suspended in glycerine, while a temperature somewhat less than 140° F. (60° C.) is sufficient to destroy it when suspended in water (p. 34). The same technique was employed when glycerine was used as the suspending medium as was employed when water was used as the medium. The same strain of virus was used in both instances. The point here illustrated is of special interest in connection with the heating of honey containing the virus of sacbrood.

HEATING REQUIRED TO DESTROY SACBROOD VIRUS WHEN SUSPENDED IN HONEY.

From the results obtained by heating the virus of sacbrood in glycerine as given above it might be expected that a higher temperature would be necessary to destroy the virus when it is suspended in honey than when it is suspended in water.

In determining the heating necessary to destroy the virus when suspended in honey the technique followed was similar to that employed when water and glycerine suspensions were used. The virus used in the inoculations bearing the date 1915 was of the same strain in all instances.

TABLE III.—*Results obtained when the virus of sacbrood was heated in honey.*

Date of inoculation.	Temperature.		Time of heating.	Results of inoculation.
	°F.	°C.	*Minutes.*	
June 1, 1915	140	60	10	Sacbrood produced.
June 11, 1915	145	63	10	Do.
Do	149	65	10	Do.
June 4, 1915	154	68	10	Do.
June 24, 1915	156	69	10	Do.
Do	158	70	10	Do.
June 1, 1915	158	70	10	No disease produced.
June 18, 1915	158	70	10	Do.
July 3, 1915	160	71	10	Do.
Aug. 28, 1915	160	71	10	Do.
Aug. 7, 1915	163	73	10	Do.
Aug. 28, 1915	163	73	10	Do.
June 1, 1915	167	75	10	Do.
Aug. 7, 1915	167	75	10	Do.
June 1, 1915	176	80	10	Do.

As shown by the results recorded in Table III, the virus of sacbrood when suspended in honey was destroyed in 10 minutes at a temperature very near 158° F. (70° C.). This temperature is more than 18° F. (10° C.) greater than the temperature required to destroy in the same time the virus when suspended in water and approximately equal to that necessary to destroy it when suspended in glycerine.

RESISTANCE OF SACBROOD VIRUS TO DRYING AT ROOM TEMPERATURE.

In the experiments made for the purpose of determining the amount of drying which the virus of sacbrood will withstand, larvæ recently dead of the disease were used. These are crushed, strained through cheesecloth, and the crushed mass poured into Petri dishes (fig. 32) to the extent of a thin layer for each dish, the material in each being the crushed remains of about 30 larvæ. These are placed in a drawer, shielding the larval material from the light. The drying then proceeds at the temperature of the room. This temperature varied greatly from day to day, sometimes being as high as 93° F. (34° C.).

At intervals, reckoned in days, after the preparation of the virus, colonies are inoculated. An aqueous suspension is made of the drying larval content contained in a Petri dish. This is added to sirup, and the sirup suspension is fed to a healthy colony. The experiments gave the following results:

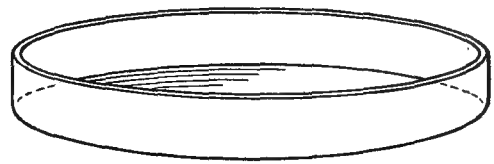


FIG. 32.—Open Petri dish. One-half of Petri dish, either top or bottom. (Original.)

TABLE IV.—*Resistance of sacbrood virus to drying at room temperature.*

Date of inoculation.	Time of drying.	Results of inoculation.
Aug. 8, 1914	3 days	Sacbrood produced.
Aug. 14, 1914	7 days	Do.
Sept. 6, 1915	13 days	Do.
July 1, 1915	16 days	Do.
Sept. 28, 1915	18 days	Do.
July 6, 1915	20 days	Do.
Sept. 3, 1915	22 days	No sacbrood produced.
Sept. 27, 1915	26 days	Do.
Oct. 9, 1914	28 days	No.
July 29, 1915	28 days	Do.
Sept. 3, 1915	35 days	Do.
Do	45 days	Do.
May 22, 1915	7 months 12 days	Do.
Do	7 months 21 days	Do.

From the results recorded in Table IV it will be noted that the virus of sacbrood in the experiment referred to withstood drying at room temperature for approximately three weeks.

The inoculations made during the third week indicated, by the reduced amount of sacbrood produced, that much of the virus had already been destroyed. Obtaining negative results from the use of larval material which had been drying more than seven months tends toward eliminating the possibility that the virus possesses a resting stage.

Similar preliminary experiments made to determine the amount of drying which the virus of sacbrood will withstand at outdoor temperature and at incubator temperature (about 99° F. [37° C.]) gave results approximately those obtained from drying at room temperature, the time being somewhat less in the case of drying at incubator temperature.

Preliminary experiments indicate also that when the virus is mixed with pollen and allowed to dry the period for which it remains virulent is increased only slightly.

RESISTANCE OF SACBROOD VIRUS TO DIRECT SUNLIGHT WHEN DRY.

In the experiments made to determine the amount of sunlight which the virus of sacbrood is capable of resisting, Petri-dish preparations similar to those made in the drying experiment were prepared. After drying a few hours in the room the uncovered dish is exposed to the direct rays of the sun. At different intervals, measured in hours, inoculations of healthy colonies are made similar to those in the drying experiments. The following results were obtained:

TABLE V.—*Resistance of the virus of sacbrood, when dry, to direct sunlight.*

Date of inoculation.	Time of exposure to sun's rays.	Results of inoculation.
	Hours.	
Sept. 17, 1915	2	Sacbrood produced.
July 29, 1915	2½	Do.
Sept. 17, 1915	3	Do.
Sept. 16, 1915	4	Do.
Do	5	Do.
Do	6	Do.
Aug. 25, 1915	6	Do.
Sept. 10, 1915	4	No disease produced.
Do	5	Do.
Sept. 9, 1915	7	Do.
Do	9	Do.
Aug. 19, 1915	12	Do.
July 16, 1915	13	Do.
Aug. 20, 1915	18	Do.
Sept. 11, 1915	21	Do.

The results recorded in Table V show that the virus of sacbrood in the experiments made was destroyed in from four to seven hours' exposure to the direct rays of the sun. The results obtained also indicate that much of the virus was destroyed in a 2-hour exposure to the sun.

It will be readily appreciated that the time that the virus will resist the sun's rays will depend a great deal upon the intensity of the rays at the time of its exposure and the thickness of the layer of the infective larval material in the Petri dish. The drying that

would naturally take place during the exposure to the sun would tend also to destroy the virus, but as the resistance to drying is better given in weeks than days, this factor may be disregarded here.

RESISTANCE OF SACBROOD VIRUS TO DIRECT SUNLIGHT WHEN SUSPENDED IN WATER.

In the experiments made for the purpose of determining the resistance of the virus of sacbrood to the direct rays of the sun when suspended in water, Petri dishes were again used. About 1½ ounces of the aqueous suspension containing the crushed tissues of 30 larvæ is poured into the dish and exposed to the direct rays of the sun. After intervals reckoned in hours the inoculations of healthy colonies are made. The contents of a single Petri dish are added to about one-half pint of sirup and the suspension fed to a healthy colony. The following results were obtained from the experiments:

TABLE VI.—*Resistance of sacbrood virus to the direct rays of the sun when suspended in water.*

Date of inoculation.	Time of exposure to sun's rays.	Results of inoculation.
	Hours.	
Sept. 10, 1915	1	Sacbrood produced.
Aug. 20, 1915	2	Do.
Sept. 14, 1915	2	Do.
Aug. 24, 1915	2	Do.
Aug. 18, 1915	3	Do.
Sept. 9, 1915	4	Do.
Sept. 10, 1915	4	Do.
Aug. 24, 1915	5	Do.
Do	4	No disease produced.
Aug. 16, 1915	5	Do.
Sept. 8, 1915	5	Do.
Do	6	Do.
Sept. 9, 1915	7	Do.
Do	8	Do.
Aug. 25, 1915	10	Do.
Aug. 20, 1915	12	Do.
July 13, 1915	13	Do.
Aug. 26, 1915	13	Do.

From Table VI it will be seen that when suspended in water the virus of sacbrood was killed in from four to six hours.

The aqueous suspensions in the Petri dishes in these experiments did not reach by several degrees the temperature 138° F. (59° C.) at which the virus is destroyed readily by heating (p. 34). Naturally experiments of the nature of those in this group will vary in all cases with the intensity of the sun's rays to which the virus is exposed. The exposures were made in these experiments between 9 and 4 o'clock, the sun's rays toward the middle of the day being most often used.

RESISTANCE OF SACBROOD VIRUS TO DIRECT SUNLIGHT WHEN SUSPENDED IN HONEY.

The crushed and strained tissue mass of larvæ dead of sacbrood was suspended in honey and exposed to the direct rays of the sun. To prevent robbing by bees, closed Petri dishes were used. At intervals reckoned in hours healthy colonies were inoculated, each with the virus from a single Petri dish. The exposures were made during the day between 9 and 4 o'clock, preference being given to the hours near midday. The group of experiments conducted on this point gave the following results:

TABLE VII.—*Resistance of the sacbrood virus to direct sunlight when suspended in honey.*

Date of inoculation.	Time of exposure to sun's rays.	Results of inoculation.
	Hours.	
Aug. 24, 1915	1	Sacbrood produced.
Do	2	Do.
Aug. 18, 1915	4	Do.
Sept. 9, 1915	4	Do.
Sept. 10, 1915	4	Do.
Aug. 24, 1915	5	Do.
Aug. 16, 1915	5	No disease produced.
Aug. 25, 1915	5	Do.
Sept. 8, 1915	5	Do.
Do	6	Do.
Sept. 9, 1915	7	Do.
Do	8	Do.
Aug. 25, 1915	10	Do.
Sept. 11, 1915	12	Do.
Aug. 26, 1915	13	Do.
Sept. 11, 1915	18	Do.

From the results of the experiments recorded in Table VII it will be observed that the virus of sacbrood when suspended in honey was destroyed by the direct rays of the sun in from five to six hours. These figures represent the time for destruction of all of the virus used in each experiment. The results obtained from the experiments indicate, however, that much of it was destroyed earlier.

LENGTH OF TIME THAT SACBROOD VIRUS REMAINS VIRULENT IN HONEY.

In devising methods for the treatment of sacbrood it is of particular interest to know the length of time that the virus will remain virulent when it is in honey. Experiments have been made to gain data on this point. Larvæ recently dead of sacbrood are crushed, strained, and suspended in honey. About one-half pint of the suspension, representing the virus from about 30 dead larvæ, is placed in each of a number of glass flasks. These are allowed to stand at room temperature, being shielded from the light by being placed in a closed cabinet.

After periods reckoned in days inoculations of healthy colonies are made. The following results have been obtained:

TABLE VIII.—*Length of time the virus of sacbrood remains virulent in honey.*

Date of inoculation.	Time virus was in honey.		Results of inoculation.
	Mos.	*Days.*	
June 17, 1915	0	20	Sacbrood produced.
June 4, 1915	0	23	Do.
Oct. 2, 1915	0	30	Do.
Sept. 3, 1915	0	24	No disease produced.
July 29, 1915	0	29	Do.
June 30, 1915	0	33	Do.
Do	0	35	Do.
July 17, 1915	0	36	Do.
Oct. 21, 1915	0	49	Do.
Sept. 8, 1915	0	70	Do.
May 13, 1915	[1] 7	10	Do.
May 6, 1915	7	20	Do.
May 4, 1915	8	2	Do.
May 18, 1915	8	21	Do.
Sept. 3, 1915	12	1	Do.

[1] The dead brown larval remains were not crushed before being introduced into the honey.

The experiments recorded in Table VIII show that the virus of sacbrood when suspended in honey at room temperature remained virulent for three weeks, but was entirely destroyed before the end of the fifth week. It is most likely that the virus in most instances is destroyed by the end of one month at this temperature.

The experiments in which the virus had been allowed to remain in the honey for more than seven months suggest that there is probably no resting stage of the virus to be considered in this connection. The facts tend to indicate that the virus does not receive any marked amount of protection by being in honey. From the dates of the experiments in this group it will be noted that the virus was subjected to summer temperature. The evidence at hand indicates that it remains virulent somewhat longer when the temperature is lower.

RESISTANCE OF SACBROOD VIRUS TO THE PRESENCE OF FERMENTATIVE PROCESSES.

Fermentation and putrefaction [1] are other means by which the virus of sacbrood may be destroyed in water. A crushed and strained mass of tissue from larvæ recently dead of the disease is suspended in a 10 per cent sugar (granulated or cane sugar) solution.

[1] "Fermentation" has reference here particularly to the breaking up of carbohydrate substances by the growth of microorganisms, the sugars in honey being naturally the carbohydrates especially of interest in these discussions. The process results in the formation of a large number of substances—acids, alcohols, etc. The odor accompanying such a process could not be called offensive. By the term "putrefaction" is meant the breaking up of nitrogenous organic substances by microorganisms. These have a chemical composition quite different from the carbohydrates. When broken up the resulting substances are more often alkaline in nature. The odor from a suspension in which putrefactive processes are going on is usually distinctly offensive.

A small quantity of soil is added to inoculate the suspension further. This is then distributed in test tubes (fig. 33), the quantity in each tube representing the virus from about 15 larvæ. These suspensions are allowed to remain at room temperature, shielded from the light. Under these conditions fermentation goes on rather rapidly.

FIG. 33.—Test tube bearing a cotton plug, used in testing the effect of fermentation, putrefaction, and disinfecting agents on the virus of sacbrood. (Original.)

After intervals reckoned in days colonies free from the disease are inoculated, each with the suspension from a single tube. Results from such inoculations are given in the following table:

TABLE IX.—*Resistance of sacbrood virus to fermentation in a 10 per cent sugar solution at room temperature.*

Date of inoculation.	Period of fermentation.	Results of inoculation.
	Days.	
Sept. 9, 1915	1	Sacbrood produced.
Sept. 11, 1915	2	Do.
Do	3	Do.
Sept. 13, 1915	4	Do.
July 14, 1915	3	No disease produced.
July 22, 1915	5	Do.
Sept. 14, 1915	5	Do.
Sept. 22, 1915	7	Do.
July 10, 1915	9	Do.
June 10, 1915	13	Do.
July 7, 1914 [1]	34	Do.
Aug. 27, 1914	51	Do.
Do	85	Do.
Do	87	Do.
Do	90	Do.
Do	244	Do.

[1] The results recorded for 1914 were obtained with a suspension of crushed larvæ, in various stages of decay, in sirup made from about equal parts water and sugar.

From the results of experiments recorded in Table IX it will be noted that the virus of sacbrood was destroyed in from three to five days in the presence of fermentation in 10 per cent cane sugar (saccharose) at room temperature.

As the rapidity of fermentative processes varies with the temperature present, it is natural to suppose that the time required for the destruction of the virus will vary. From experiments it is found that at incubator temperature the time is slightly less, and at outdoor temperature it is somewhat greater than at room temperature.

RESISTANCE OF SACBROOD VIRUS TO FERMENTATION IN DILUTED HONEY AT OUTDOOR TEMPERATURE.

Employing the egg test [1] as used by beekeepers in diluting honey for the purpose of making vinegar, it is found that it requires about

[1] This test is applied in the following manner: Water is added to honey until an egg placed in the mixture is nearly submerged, the surface remaining above the liquid being only about as large as a 10-cent piece.

four volumes of water to one of ripened honey to obtain the strength recommended. The honey solution by volume, therefore, is about 20 per cent honey.

A suspension of the virus of sacbrood in such a solution is distributed in test tubes placed in an empty hive body and allowed to ferment at outdoor temperature. After periods reckoned in days colonies are inoculated as was done in case of the sugar solutions described above. The following results were obtained from the experiments performed:

TABLE X.—*Resistance of sacbrood virus to fermentative processes in a 20 per cent honey solution at outdoor temperature.*

Date of inoculation.	Time of fermentation.	Results of inoculation.
	Days.	
Sept. 11, 1915	3	Sacbrood produced.
Sept. 13, 1915	4	Do.
Sept. 14, 1915	5	No disease produced.
Aug. 4, 1915	6	Do.
Sept. 15, 1915	6	Do.
Sept. 14, 1915	7	Do.
Sept. 22, 1915	7	Do.
Sept. 17, 1915	8	Do.
Sept. 8, 1915	10	Do.

In the presence of fermentative processes taking place in a 20 per cent honey solution at outdoor temperature it will be observed that the virus of sacbrood in the experiments recorded in Table X was destroyed in six days. The outdoor temperature during these experiments was quite warm. Had it been cooler, the time for the destruction of the virus would have been somewhat increased. In the making of vinegar it may be concluded that the virus of sacbrood, should it be present in the honey used, would be destroyed in a comparatively short time as a result of fermentation.

RESISTANCE OF SACBROOD VIRUS TO THE PRESENCE OF PUTREFACTIVE PROCESSES.

Larvæ containing the virus of sacbrood are crushed and suspended in water. A small quantity of soil is added. The suspension is strained and distributed in test tubes. These are allowed to stand at room temperature in a state of putrefaction. After periods reckoned in days colonies free from the disease are inoculated, each with the contents of a single tube added to sirup. From experiments of this kind the results following have been obtained.

TABLE XI.—*Resistance of sacbrood virus to putrefaction.*

Date of inoculation.	Time of putrefaction.	Results of inoculation.
	Days.	
Aug. 6, 1914	1	Sacbrood produced.
Aug. 7, 1914	2	Do.
Aug. 10, 1914	3	Do.
July 20, 1915	3	Do.
Sept. 13, 1915	4	Do.
Sept. 14, 1915	5	Do.
July 22, 1915	5	Do.
July 8, 1915	7	Do.
May 22, 1915	9	Do.
Sept. 22, 1915	7	No disease produced.
Aug. 18, 1915	10	Do.
Sept. 16, 1914	14	Do.
Sept. 25, 1914	14	Do.
July 1, 1915	16	Do.

From Table XI it will be noted that the virus of sacbrood was destroyed in the experiments recorded in from 7 to 10 days. As in the case of fermentation, so in the case of putrefaction, it is to be expected that the time for the destruction of the virus will vary appreciably with the temperature at which the putrefactive processes take place.

RESISTANCE OF SACBROOD VIRUS TO CARBOLIC ACID.

Larvæ recently dead of sacbrood are crushed and strained. This larval mass is diluted with carbolic acid in aqueous solution. About 10 parts of carbolic acid to 1 part of the larval mass is used. This suspension is distributed in test tubes and allowed to stand at room temperature. Each tube contains the virus from about 15 larvæ. After periods, reckoned in days, colonies free from disease are inoculated, each with the contents of a single tube added to sirup.

Carbolic acid solutions of $\frac{1}{2}$, 1, 2, and 4 per cent were used in making the suspensions. The following results were obtained from the experiments:

TABLE XII.—*Resistance of sacbrood virus to carbolic acid.*

Date of inoculation.	Strength of carbolic acid used.	Time in suspension.	Results of inoculation.
	Per cent.	*Days.*	
Sept. 3, 1914	$\frac{1}{2}$	1	Sacbrood produced.
Sept. 18, 1914	$\frac{1}{2}$	16	Do.
Sept. 3, 1914	$\frac{1}{2}$	24	Do.
Sept. 17, 1914	$\frac{1}{2}$	38	No disease produced.
Aug. 12, 1915	$\frac{1}{2}$	50	Do.
Aug. 20, 1915	$\frac{1}{2}$	50	Do.
May 14, 1915	$\frac{1}{2}$	238	Do.
Sept. 3, 1914	1	1	Sacbrood produced.
Sept. 18, 1914	1	16	Do.
June 23, 1915	1	25	Do.
Sept. 17, 1915	1	38	No disease produced.
Aug. 12, 1915	1	50	Do.
Aug. 21, 1915	1	50	Do.
June 4, 1915	1	251	Do.

TABLE XII.—*Resistance of sacbrood virus to carbolic acid*—Continued.

Date of inoculation.	Strength of carbolic acid used.	Time in suspension.	Results of inoculation.
	Per cent.	*Days.*	
Sept. 3, 1914	2	1	Sacbrood produced.
Sept. 18, 1914	2	16	Do.
June 23, 1915	2	25	Do.
Sept. 17, 1915	2	38	No disease produced.
Aug. 12, 1915	2	42	Do.
Aug. 21, 1915	2	50	Do.
		Hours.	
June 23, 1915	4	3	Sacbrood produced.
July 1, 1915	4	7	Do.
		Days.	
June 23, 1915	4	25	No disease produced.
Aug. 12, 1915	4	50	Do.

From the preliminary results recorded in Table XII it will be observed that the virus of sacbrood shows a marked resistance to the disinfecting power of carbolic acid. Under the conditions of the experiments the virus resisted its action for more than three weeks in ½, 1, and 2 per cent aqueous solutions.

These results lead naturally to a consideration of the effect of drugs on the virus of sacbrood in the treatment of the disease. On this point complete data are yet wanting.

While the disinfecting power of a compound, as shown in experiments such as those described above for carbolic acid, may indicate something as to the value of the compound as a drug, it does not necessarily prove its value. More definite proof is gained through feeding colonies with the virus suspended in honey medicated with the drug, and then continuing to feed the inoculated colonies with honey similarly medicated daily thereafter until the time for the appearance of the disease.

To illustrate the nature of experiments which are being conducted to determine the value of drugs in the treatment of sacbrood, experiments with quinine and carbolic acid are here referred to. A colony was fed the virus of sacbrood suspended in honey and water, equal parts, to which was added 5 grains of the bisulphate of quinine to one-half pint of diluted honey, and on each of the five days following the inoculation the same colony was fed diluted honey containing no virus, but medicated with quinine in the same way. On the seventh day following the inoculation with the virus there was found to be a large quantity of sacbrood produced in the colony so inoculated and treated.

A similar experiment in which carbolized honey was used gave like results. These experiments, although not furnishing conclusive proof, do indicate something of what might be expected from the use of quinine or carbolic acid as a drug in the treatment of sacbrood.

Technically the foregoing studies should be thought of as being preliminary. Questions relating to virulence of the virus, resistance of the bees, technique, and many other factors contribute to make results such as these vary. For practical purposes, however, they are sufficiently complete. In estimating the time necessary for the destruction of the virus in practical apiculture by any of the foregoing tables of results it should be emphasized that the time element should be somewhat increased, inasmuch as the conditions present in the experiments were more favorable for its destruction than would ordinarily be the case in practice.

MODES OF TRANSMISSION OF SACBROOD.

The transmission of a brood disease must be thought of as taking place (1) from diseased to healthy brood within a colony and (2) from a diseased colony to a healthy one. The manner in which sacbrood is spread naturally depends directly upon the modes by which the virus of the disease is transmitted.

As is shown experimentally, the virus of sacbrood produces the disease when it is added directly to the food of young larvæ or when it is mixed with sirup and fed to a colony. From this fact it is fair to assume that sacbrood may result whenever the food or water used by the bees contains the living virus of the disease.

Bees have a tendency to remove diseased or dead larvæ from the cells. When the removal is attempted about the time of death, it is done piecemeal. Each fragment removed from such a larva, if fed to a young healthy larva within a week, would most likely produce sacbrood in the larva. Within the hive, therefore, the disease may be transmitted to healthy larvæ more or less directly in this way.

Just what becomes of these bits of tissue removed from the diseased larvæ, however, is not known. If it were the rule that the tissues of the dead larva after being removed in fragments were fed unaltered to the young healthy larvæ within two weeks after its removal, it would seem that the disease would increase rapidly in the colony as a result. Such an increase, however, is unusual, the tendency in a colony being in most cases toward a recovery from the disease.

This fact leads one to think of other possibilities regarding the destiny of the infected tissues removed as fragments from the diseased larvæ. If the infective material were fed to the older larvæ, death probably would not result. Should it be used by adult bees as food for themselves, the likelihood of the transmission of the disease under such circumstances would apparently be very materially reduced. If the infective material were stored with the honey and

did not reach the brood within a month or six weeks, it is not probable that the disease would be transmitted under such circumstances (p. 41). Should the dead larvæ or any fragments of them be carried out of the hive, the virus would have to be returned to the hive, as a matter of course, before further infection of the brood could take place from such infective material.

It is left to be considered in what way the infective material if removed from the hive might be returned to the brood and infect it. Should any material containing the virus reach the water supply of the bees, or the flowers visited by the bees, it is within the range of possibility that some of the living virus might be returned to the hive and reach healthy young larvæ.

While out of the hive, however, the virus must withstand certain destructive agencies in nature. Under more or less favorable circumstances it would withstand drying alone for a few weeks (p. 37), but if exposed to the sun it might be destroyed in a few hours. (p 38). If the virus were subjected to fermentation it might be destroyed within a week (p 43), and if subjected to putrefaction, within two weeks (p. 44).

The experimental evidence indicates that the virus, once out of the hive and freed from the adult bees removing it, during the warmer seasons of the year, at least, has but little chance of being returned to the hive and producing any noticeable infection. In the experimental apiary (Pl. III) a large number of colonies have been heavily infected with sacbrood through experimental inoculation; and no infection was observed to have resulted in the uninoculated colonies. If throughout the main brood-rearing season the usual source of infection were the flowers or the water supply, a quite different result would be expected.

Tentatively it may be concluded, therefore, that the probability of the transmission of the virus of sacbrood by way of flowers visited by bees, practically considered, is quite remote, being, however, to a limited extent theoretically possible.

It would seem that there is a greater likelihood of the water supply being a source of infection than flowers. The chances for infection from this source, should it occur at all, would be greater in the spring, as at such a time the quantity of infective material in diseased colonies is greater, increasing the chances that some of it might be carried to the water supply and contaminate it, and furthermore, the destructive agencies in nature are at this time less efficient.

Bees drifting or straying from infected colonies to healthy ones must be thought of as possible transmitters of the disease. That the disease is not spread to any great extent in this way is evidenced

by the fact that colonies in the apiary that were not inoculated experimentally remained free from disease, although many colonies in the apiary were heavily infected at the time.

Sacbrood has a tendency to weaken a colony in which it is present. Frequently this weakness is noticeable and often marked. Robbing, which occurs not infrequently at such a time, results in the transmission of the virus, to some extent at least, directly to healthy colonies. Robbing, therefore, must always be considered as a probable means of transmission.

The modes of transmission of sacbrood within the colony and from colony to colony, as will be seen, are not by any means completely determined. In what way the sacbrood virus is carried over from one brood-rearing season to another is one of the many problems concerning this disease that are yet to be solved. The foregoing facts, accompanied by the brief discussions, it is hoped, will throw some light upon this important phase of the study—the transmission of this disease—and will serve as an aid to later researches.

DIAGNOSIS OF SACBROOD.

The diagnosis of sacbrood can be made from the symptoms already described (p. 10). The colony may or may not be noticeably weakened. The adult bees are normal in appearance. Scattered here and there on the brood frame among the healthy brood are found dead larvæ in the late larval stage. Usually there are only a few of them, yet sometimes there are many. These larvæ may be in capped or uncapped cells. When found in uncapped cells, however, the cappings had already been removed by the bees after the death of the larvæ. The cap over a dead larva in a cell may be found punctured or not. The brood possesses no abnormal odor, or practically none.

The post-mortem appearances of larvæ dead of the disease are especially valuable in making the diagnosis. The larva is found extended lengthwise in the cell and on its dorsal side. Throughout the period of decay it will be found to maintain much of the form and markings of a healthy larva of the age at which it died. Soon after death the larval remains are slightly yellow. After a period they assume a brownish tint. Since the brown color deepens as the process of decay and drying takes place, the remains may be found having any one of a number of shades of brown. They may appear at times almost black.

After death the cuticular portion of the body wall becomes toughened, permitting the easy removal of the larva intact from the cell. When removed, the saclike appearance of the remains becomes easily apparent. Upon rupturing the cuticular sac the contents are found to be a brownish, granular-appearing mass suspended in a compara-

tively small quantity of more or less clear liquid. The scales formed by the drying of the decaying remains are easily removed from the cells. After becoming quite dry many of them indeed can be shaken from the brood comb.

Upon crushing larvæ which have been found dead for some time but not yet dry, a marked unpleasant odor will be noticed if the crushed mass is held near the nostrils.

Microscopically no microorganisms are to be found in the decaying remains of the larvæ. Cultures made from them are also negative.

Differential diagnosis.—Sacbrood must be differentiated from the other brood diseases.

American foulbrood may be recognized by the peculiar odor of the brood combs when the odor is present. The body wall of the larval and pupal remains is easily ruptured, and the decaying mass becomes viscid, giving the appearance popularly referred to as "ropiness." The scale adheres quite firmly to the floor of the cell. The presence of *Bacillus larvæ* in the brood dead of the disease is a positive means by which it may be differentiated from sacbrood.

European foulbrood may be recognized by the fact that the larvæ as a rule die while coiled in the cell and before an endwise position is assumed. In the majority of instances, therefore, death takes place before the cells are capped. The saclike appearance characterizing the dead larvæ in sacbrood is absent. The granular consistency of the decaying mass is absent also. Microscopically, a large number of bacteria are found in larvæ dead of European foulbrood, but are absent in larvæ dead of sacbrood. The presence of *Bacillus pluton* is a positive means by which European foulbrood may be recognized. *Bacillus alvei* and other species may also be present.

Sacbrood must also be differentiated from other conditions referred to as chilled brood, overheated brood, and starved brood, which occasionally are encountered. This can be done by a comparison of the symptoms presented by these different conditions with the symptoms of sacbrood, and the history of the cases. Some of the larvæ dead from these conditions will be found to have died while yet coiled in the cell. This fact suggests some condition other than sacbrood. When dying later, the saclike remains characterizing sacbrood are not present in conditions other than sacbrood.

PROGNOSIS.

The tendency in a colony affected with sacbrood is to recover from the disease. Colonies which during the spring months show the presence of more or less disease, by midsummer or earlier may, and very

frequently do, contain no diseased brood. Experimentally it is possible to destroy a colony by feeding it repeatedly the virus of sacbrood, and beekeepers report that the disease sometimes destroys colonies in their apiaries. The percentage of colonies, however, that actually die out as a direct result of the disease is small. The weakening of the colony in the spring of the year not only reduces or entirely eliminates the profits on it for the season, but may also cause it to be in a weakened condition on the approach of winter.

Whether a larva once infected ever recovers from the disease is not known. Reasoning from what is known of the diseases of other animals and man, one would expect that a larva may recover from sacbrood infection. It is known that many larvæ, both worker and drone, do die. From the information thus far obtained it does not appear that a queenless colony would be likely to remain so as a consequence of the disease.

As to the prognosis of the disease in a colony it may be said, therefore, that it is very favorable for the continued existence of the colony. As to the economic losses to be expected from the disease, the present studies suggest that they may vary from losses that are so light as not to be detected upon examination to losses that may equal the entire profits of the colony for the year. Indeed, at times the death of the colony takes place as a result of the disease.

RELATION OF THESE STUDIES TO THE TREATMENT OF SACBROOD.

An earlier paper (White, 1908) contains a brief general discussion of the relation existing between the cause of bee diseases and the treatment of them. The general remarks made in it apply also to sacbrood. No doubt the beekeeper in studying the results given here has already observed relations existing between them and points which should be incorporated in methods for treatment. Mentioning a few of them here may serve to suggest still others.

That the weakness resulting in a sacbrood colony is due to the death of worker larvæ; that adult bees are not susceptible to the disease; that queenlessness is rarely to be expected as a sequence of the disease; that the disease may be produced with ease at any time of the year that brood is being reared; that it occurs at all seasons, but is more frequently encountered in the spring; that it is found in localities differing widely as to food and climatic conditions; and that no complete racial immunity to the disease has yet been found are facts concerning the predisposing causes of sacbrood which beekeepers will at once recognize as bearing a close relation to the methods by which the disease should be treated.

As sacbrood can not occur in the absence of its exciting cause (a filterable virus), a knowledge of this cause is of special importance in the treatment of the disease.

That sacbrood is very frequently encountered; that it is infectious, but that it is more benign in character than malignant; that it does not spread rapidly from one colony to another; that colonies manifest a strong tendency toward self-recovery from the disease; that this tendency is stronger after midsummer; that the disease may so weaken a colony during the early brood-rearing season that the profits from it may be much reduced, or even rendered nil; and that the disease may indeed destroy the colony are facts which must be considered in devising logical methods for its treatment.

That the virus of sacbrood remains virulent in larvæ dead of the disease for less than one month; that it remains virulent in honey approximately one month; that when mixed with pollen it ceases to be virulent after about one month; and that in drying no virulence is to be expected after one month, are facts that account in a large measure for the strong tendency to recover from the disease manifested by the colony and that furnish information concerning the use of combs from sacbrood colonies. From the results it may be concluded that it is better, theoretically, to store combs from sacbrood colonies for one or two months before they are again used, provided such storing entails no particular inconvenience or financial loss to the beekeeper.

Further experiments show that brood frames from badly-infected colonies may be inserted into strong, healthy ones, and cause thereby very little infection and consequently only a slight loss. This is especially true after the early brood-rearing season of the year is past. Since this can be done, it is quite probable that the practical beekeeper will find that this disposition of the combs will be the preferable one to make. At any event, it is comforting to know that it is never necessary to destroy the combs from sacbrood colonies on account of the disease.

The experimental results here given regarding the destruction of the virus through heating, fermentation, putrefaction, drying, and direct sunlight should assist materially in the solution of the problem of the transmission of sacbrood, and should be found helpful in devising efficient methods for the treatment of the disease.

Toward disinfecting agents it is shown that the virus of sacbrood possesses, in some instances at least, marked resistance. These and other experimental results thus far obtained indicate that the use of any drug in the treatment of the disease should not be depended upon until such a drug has been proved to be of value.

No fear need be entertained in practical apiculture that the disease will be transmitted by the hands or clothing of the operator, by the tools used about the apiary, through the medium of the wind, or by the queen. It would seem at all times superfluous in the case of sacbrood to flame or burn the inside of the hive or to treat the ground about a hive containing an infected colony.

There is but little danger that the disease will be transmitted by way of flowers visited by bees from sacbrood colonies and later from healthy ones.

Theoretically, it is possible that the disease may be transmitted through a contamination of the water supply by bees from sacbrood colonies. Whether infection ever takes place in this way, however, is not yet known. If the disease is ever transmitted in this way, it would seem that it is more likely to take place in the spring of the year than at any other season.

While there is yet much to be learned about sacbrood, it is hoped that by carefully considering these studies the beekeepers will be aided in devising efficient and economical methods for its treatment.

SUMMARY AND CONCLUSIONS.

The following summary and statements of conclusions seem to be justified as a result of the investigations recorded in this paper:

(1) Sacbrood is an infectious disease of the brood of bees.

(2) Adult bees are not susceptible to the disease.

(3) The infecting agent causing sacbrood is of such a nature that it passes through the pores of a fine clay filter. It is therefore a filterable virus.

(4) A colony may be inoculated by feeding it sirup or honey containing the virus.

(5) The quantity of virus contained in a single larva recently dead of the disease is sufficient to produce quite a large amount of sacbrood in a colony.

(6) The period from time of inoculation to the appearance of the first symptoms of the disease—the incubation period—is approximately six days, being frequently slightly less.

(7) By inoculation the disease may be produced at any season of the year that brood is being reared.

(8) The disease is more often encountered during the first half of the brood-rearing season than during the second half.

(9) It occurs among bees in localities having as wide a range of climatic conditions, at least, as are found in the United States.

(10) The course of the disease is not greatly affected by the character or quantity of the food obtained and used by the bees.

(11) Larval remains recently dead of the disease prove to be very infectious when fed to bees. Dead larvæ which have been in the brood comb more than one month are apparently noninfectious.

(12) Colonies possess a strong tendency to recover from the disease without treatment.

(13) The virus of sacbrood suspended in water and heated to 138° F. (59° C.) was destroyed in 10 minutes. Considering the varying factors which enter into the problem, the minimum temperature necessary to destroy this virus when applied for 10 minutes should

be found at all times to lie somewhere between the limits of 131° F. (55° C.) and 149° F. (65° C.).

(14) When the virus of sacbrood is susponded in honey it may be destroyed by heating the suspension for 10 minutes at approximately 158° F. (70° C.).

(15) The virus resisted drying at room temperature for approximately three weeks.

(16) The virus when dry was destroyed by the direct rays of the sun in from four to seven hours.

(17) The virus when suspended in water was destroyed by the direct rays of the sun in from four to six hours.

(18) The virus when suspended in honey was destroyed by the direct rays of the sun in from five to six hours.

(19) The virus when suspended in honey and shielded from direct sunlight remained virulent for slightly less than one month at room temperature during the summer.

(20) The virus was destroyed in approximately five days in the presence of fermentative processes taking place in 10 per cent sugar solution at room temperature.

(21) In the presence of fermentative processes going on in 20 per cent honey solution at outdoor temperature the virus of sacbrood was destroyed in approximately five days.

(22) In the presence of putrefactive processes the virus remained virulent for approximately 10 days.

(23) The virus will resist ½ per cent, 1 per cent, and 2 per cent aqueous solutions of carbolic acid, respectively, for more than three weeks, 4 per cent being more effective.

(24) Neither carbolic acid nor quinine as drugs should at present be relied upon in the treatment of sacbrood.

(25) Varying factors entering into many of the problems discussed in this paper tend to vary the results obtained. In such problems the results here given must be considered from a technical point of view as being approximate only. They are sufficiently exact for application by the beekeeper, but to insure the destruction of the virus in practical apiculture the time element indicated from these experiments as sufficient should be increased somewhat.

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